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A NEW SEED MIXER AND SAMPLER[1]

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During recent months a laboratory seed mixer and sampler, based on what is believed to be a new principle, has been developed in the Seed Research Laboratory, Division of Plant Products, Dominion Department of Agriculture.

Figures 1 to 4 give the details of its design and construction. Dimensions have not been given because they may be varied to suit the size of seed and the quantity to be mixed. The principle may also be adapted to the large scale bulking of seeds.

In operation, the seed is fed into the hopper at the top and falls by gravity over the series of dividers and baffles, being alternately divided and mixed as many times as there are sets of baffles; 9 were used in the case of the experimental machine already made. The mixed seed issues at two spouts into covered seed containers.

The hopper is provided with an adjustable sliding gate to regulate the flow of seed. For the most efficient action the gate is adjusted to suit the kind of seed being mixed. To indicate the adjustment a pointer is provided as shown in Figure 2. The gate should be opened only far enough to allow a smooth and uninterrupted flow of seeds.

A short distance below the first pair of baffles is an eccentric pin as shown on Figures 1 and 2. With certain seeds, e.g. Kentucky bluegrass, there is a tendency for the flow of falling seed to be heavier on one side than the other. The pin, being eccentric, serves to break this flow, and may be adjusted to distribute the seed evenly. Even delivery to the seed containers below the spouts is an indication that the machine is working satisfactorily.

In construction, the general proportions, as shown, should be carefully observed, the angles of the baffles and knife-edges should be accurately set, and there should be no rough finish on which seeds may lodge. The knife-edges should be accurately centred and the symmetry of the whole should be as nearly perfect as possible. The glass front should be of "ordinary" glass; glasses or glass substitutes of high dielectric quality are unsuitable. The glass front is readily removable for cleaning.

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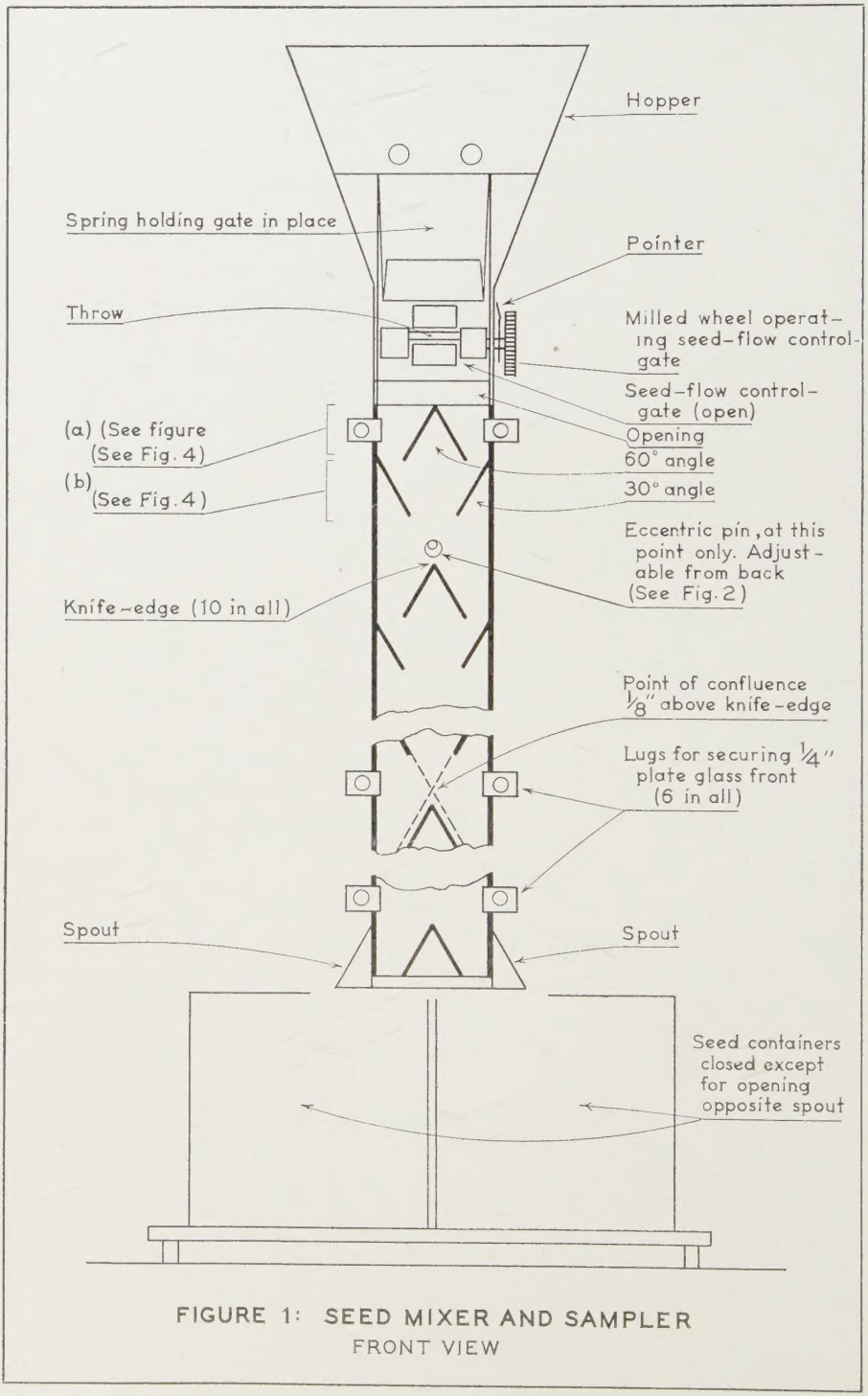

FIGURE 1: SEED MIXER AND SAMPLER
FRONT VIEW

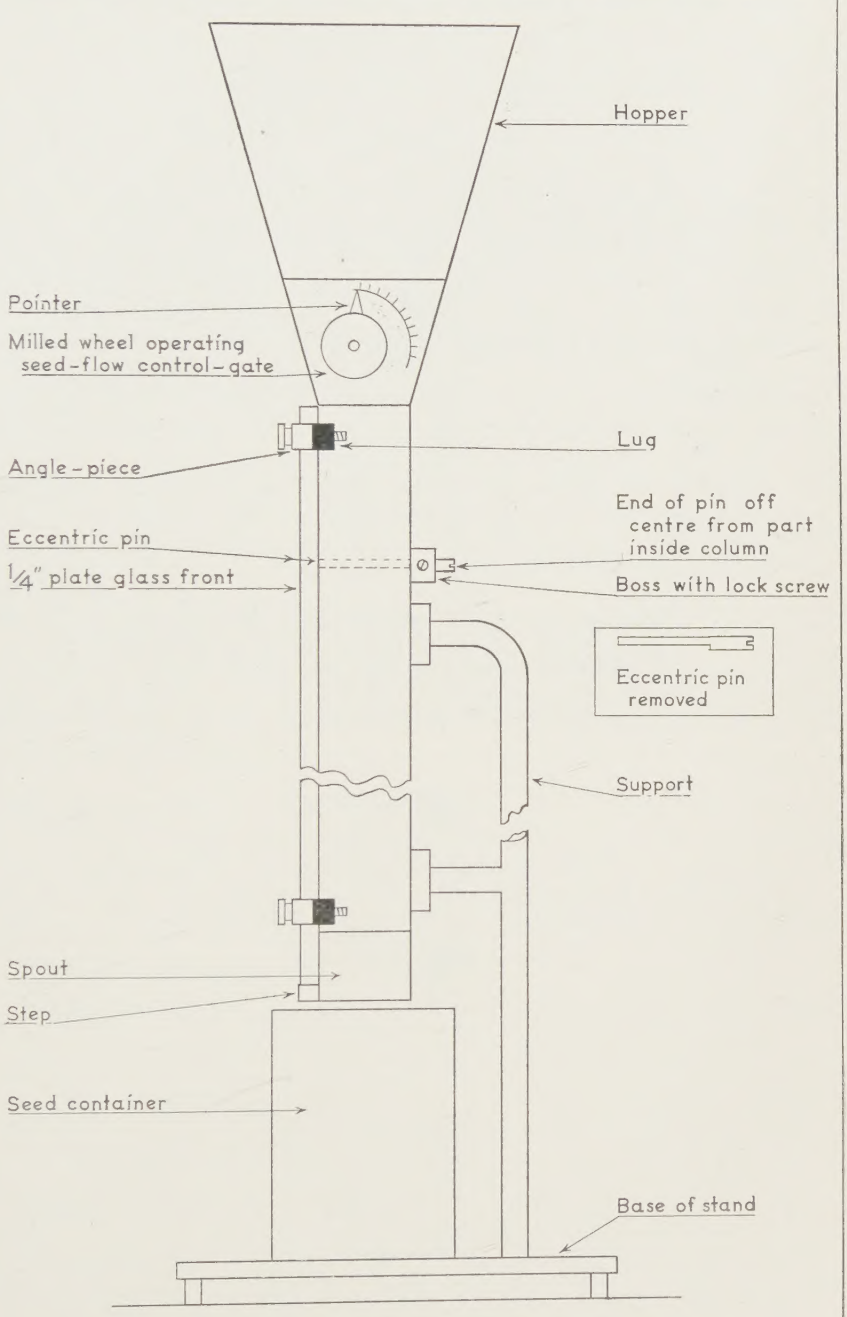

FIGURE 2: SEED MIXER AND SAMPLER
VIEW FROM RIGHT SIDE

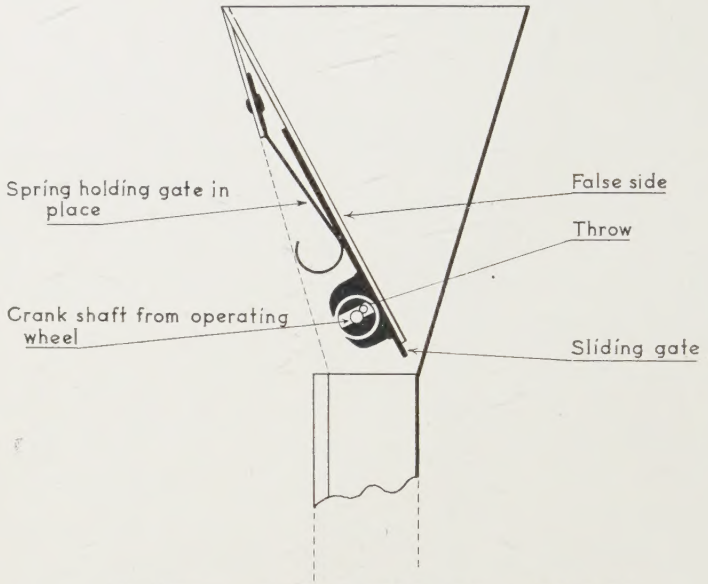

FIGURE 3: SEED MIXER AND SAMPLER
DETAILS OF CONTROL GATE. VIEW OF HOPPER RIGHT SIDE REMOVED, SHOWING MECHANISM.

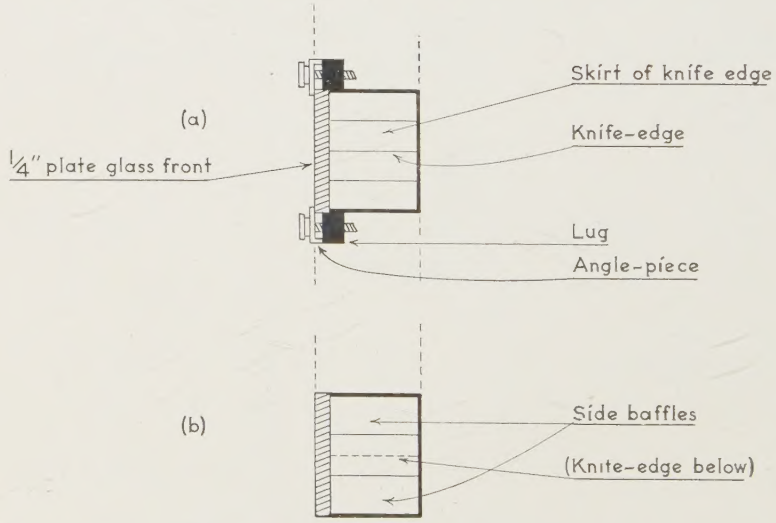

FIGURE 4: SEED MIXER AND SAMPLER
CROSS SECTIONS AT POINTS (a) AND (b)
(SEE FIGURE 1)

EXPERIMENTS ON OLD BLUEGRASS PASTURE[1]

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The object in laying down the following experiments in 1930 was to determine the yield and chemical and botanical composition of a typical Ontario bluegrass pasture of long standing under different fertilizer and cutting treatments. Previous to 1930 very little data had been gathered on the yield of natural bluegrass pastures in Ontario. This study furnished the basis of comparing the yield and nutritive value of bluegrass pasture with other farm crops.

The following experiments were carried out on a typical old Ontario bluegrass pasture which had been grazed for 25 years without fertilizing. The pasture is located on the Stone farm near Guelph. The soil is a Bellfontaine loam underlaid with gravel. It is alkaline in reaction with a pH of 7·8, and is low in available phosphorus, showing 39 p.p.m. Botanical analyses of the original sward at different seasons were made by the quadrat method. The sward presents a close association of the grasses and clovers with a sprinkling of weeds.

The dominant species are Kentucky blue 64%, white clover, yellow trefoil, twitch; secondary dominants are timothy, meadow fescue, Canadian blue, red top, with traces of red clover, alsike and weeds (chiefly chickweed, dandelion, ox-eye daisy, field bindweed, buttercup, plantain, crawfoot, Canada thistle and wild carrot).

EXPERIMENT 1

An effort was made to determine the influence of different lengths of rest period on yield, botanical composition, and chemical composition of old bluegrass pasture, with and without fertilizers. The botanical composition was determined by actual separation and weighing of the different components, e.g.: grasses, clovers, and weeds.

TABLE 1.—EXPERIMENT 1. INFLUENCE OF DIFFERENT PERIODS OF REST ON BLUEGRASS PASTURE—AVERAGE 3 YEARS

Intervals of rest	Fertilizer treatment	Dry matter per acre	Clovers in flora	Ash per acre	Calcium per acre	Phosphorus per acre	Crude protein per acre
		lb.	%	lb.	lb.	lb.	lb.
Cut weekly	Nil	1593	29	128.87	12.76	4.73	410.99
Cut fortnightly	Nil	1697	32	143.23	13.36	5.07	406.09
Cut monthly (5)*	Nil	1663	30	136.03	12.85	5.00	392.97
Cut monthly (5)	NPK	2237	12	176.72	13.29	6.46	497.96
Cut May to July (2)	Nil	1287	26	105.28	9.96	3.50	275.03
Cut May to Aug. (3)	Nil	1583	23	127.11	12.89	4.42	336.55
Cut May to Sept. (4)	Nil	1617	33	135.67	16.36	3.91	347.17
Cut July to Sept. (3)	Nil	2153	33	169.44	17.31	5.08	428.23

[1] Contribution from the Field Husbandry Department, Ontario Agricultural College, Guelph, Ontario.
[2] Associate Professor of Field Husbandry.
* Number of months under cutting.

The experimental area was laid out in 4 randomized blocks in which each treatment was replicated 4 times. Individual plots were each 10 × 10 links or 1/1000 acre in area. The same plan of randomization was used in Experiments 1, 2, and 3. Cuttings were made each month for 5 months with a lawn mower and composite samples were taken for analysis.

The fertilizer treatment for Experiments 1, 2, and 3 was at the following rates per acre: 625 lb. of 16% phosphate, and 100 lb. of potash applied every 4 years; one hundred pounds of ammonium sulphate was applied monthly for 4 months annually, equivalent to 80 lb. of nitrogen.

Conclusions—Experiment 1

1. The application of N.P.K. gives a significant increase in total yield of dry matter, protein and minerals over no treatment, but the percentage of clovers is significantly lower owing to the influence of nitrogen.

2. Frequent defoliation, i.e. weekly cutting, gives the highest percentage of protein and the lowest percentage of crude fibre.

3. The percentage of protein decreases with longer intervals of rest, while the percentage of fibre increases.

4. The total yield of dry matter for the first 2 months, May and June, is 77·39% of the total yield of 1663 lb. for 5 months.

This characteristic high curve of production of Kentucky blue in June reflects the physiological response of this species in the Ontario habitat.

The seasonal production curve of Kentucky blue grass has far reaching economic influences on dairy and livestock production, because the average milk flow, rate of growth and gain of beef cattle is correlated with this downward curve of production from Kentucky blue grass, showing a 40% drop in the dry period of rapid evaporation in July and August, when Kentucky blue goes into a dormant, unproductive stage. This critical period presents one of the major problems of pasture production in Ontario.

5. The highest yield of dry matter, 2153 lb., and of protein 428 lb., was obtained by resting the pasture in May and June. The digestion coefficient of this late cut material has been shown to be lower than the less mature forage.

EXPERIMENT 2

The object of this experiment was to determine the influence of different heights of cutting on yield, botanical composition, and chemical composition of blue grass pasture with and without fertilizers.

Conclusions—Experiment 2

1. Yield of dry matter increased progressively with increase in height of cutting.

2. The percentage of crude protein decreased with increased height.

3. Total crude protein increased progressively with increase in height.

4. The application of N.P.K. gave significant increases in yield of dry matter over control for each height.

5. The clovers were suppressed by the influence of the nitrogen carrier.

TABLE 2.—EXPERIMENT 2. INFLUENCE OF CUTTING BLUEGRASS PASTURE AT VARYING LENGTHS—AVERAGE 3 YEARS

Heights of cutting	Fertilizer treatment	Dry matter per acre	Clovers in flora	Ash per acre	Calcium per acre	Phosphorus per acre	Crude protein per acre
		lb.	%	lb.	lb.	lb.	lb.
3 inches	Nil	1383	26	105.11	10.22	3.96	282.96
3 inches	NPK	1997	16	155.57	18.27	6.53	405.19
6 inches	Nil	1700	22	125.29	12.14	5.00	287.64
6 inches	NPK	2210	17	154.48	16.93	6.74	394.04
9 inches	Nil	2400	19	154.08	19.44	4.75	349.44
9 inches	NPK	2873	14	191.63	21.89	6.72	440.72

EXPERIMENT 3

The object of this experiment was to determine the influence of cutting at different intervals of time, e.g. 10 days, 30 days, 40 days, on yield, botanical composition, and chemical composition of blue grass pasture, with and without fertilizers.

TABLE 3.—EXPERIMENT 3. INFLUENCE OF CUTTING BLUEGRASS PASTURE AT 10–30–40 DAY INTERVALS—AVERAGE 3 YEARS

Intervals of cutting	Fertilizer treatment	Dry matter per acre	Clovers in flora	Ash per acre	Calcium per acre	Phosphorus per acre	Crude protein per acre
		lb.	%	lb.	lb.	lb.	lb.
10 days	Nil	1513	35	130.27	12.78	4.86	377.80
10 days	NPK	2307	15	206.48	16.15	8.56	591.05
30 days	Nil	1680	34	154.56	15.78	5.28	361.03
30 days	NPK	2490	16	194.72	16.28	8.07	520.16
40 days	Nil	1967	32	165.03	16.98	5.76	419.56
40 days	NPK	2360	19	196.59	18.34	7.15	509.76

CONCLUSIONS—EXPERIMENT 3

1. The data show that there is a progressive increase in yield of dry matter, minerals and crude protein with increasing intervals of rest from 10 up to 40 days.

2. The percentage of crude protein is highest, i.e. 24·97% when cut every 10 days, decreasing to 21·33% when cut every 40 days.

EXPERIMENT 4

The object of this experiment was to determine the influence of different organic and inorganic fertilizers and combinations of fertilizers on yield, botanical composition, and chemical composition of old blue grass pasture.

Plots 1/20 acre in area were laid out and a section of these was fenced in which a 1/100 acre area was cut each month for 5 months. The remainder of the plots were left under natural grazing conditions to study the influence of grazing on the flora.

The fertilizer treatments were as follows: minerals applied every 4 years at the following rates per acre, 625 lb. of 16% phosphate, 100 lb. of potash, 1000 lb. of lime on Plots 1 and 8. Nitrogen carriers were applied as follows: 100 lb. per acre of ammonium sulphate each month for 4 months, or 400 lb. annually equal to 80 lb. of nitrogen. The applications of the other nitrogen carriers and manure were equated to 80 lb. of nitrogen.

TABLE 4.—EXPERIMENT 4. THE INFLUENCE OF DIFFERENT MANURES ON THE YIELD, BOTANICAL AND CHEMICAL COMPOSITION OF A 25-YEAR OLD BLUEGRASS PASTURE. STONE FARM. AVERAGE 3 YEARS

Plot	Fertilizer treatment	Dry matter per acre	Clover	Ash per acre	Calcium per acre	Phosphorus per acre	Crude protein per acre
		lb.	%	lb.	lb.	lb.	lb.
1	Ca NPK	2544	6.0	194	22.24	7.62	569
2	NPK	2552	6.7	188	22.02	9.36	562
3	NP	2364	7.4	175	23.23	8.56	537
4	NK	2502	5.2	182	24.54	8.44	572
5	PK	1880	21.7	152	25.47	7.54	411
6	Check	1653	24.2	129	19.46	5.78	368
7	N	2198	5.9	155	20.74	7.00	503
8	Ca	1636	14.2	126	19.37	5.88	346
9	K	1950	17.3	167	24.13	7.30	435
10	P	2142	20.3	188	23.82	9.08	470
11*	PK ($NaNO_3$)	3269	7.1	292	30.29	13.28	697
12*	PK (Nitro-chalk)	3387	7.1	314	32.25	16.53	733
13*	Barnyard manure	2820	8.4	232	26.02	11.65	639
14*	Liquid manure	2281	7.9	185	19.74	9.22	553

*Average 2 years.

CONCLUSIONS—EXPERIMENT 4

1. The influence of the nitrogen carrying manures on depressing the clovers was marked, whereas the minerals or combinations of minerals tended to maintain the percentage and balance of legumes found in the natural sward which had reached a competitive equilibrium.

2. The nitrogen carrying manures depressed the percentage of calcium in the fodder.

3. The addition of calcium on this alkaline soil did not increase yield, but tended to depress yield especially in the first year of application.

4. The readily available nitrogen carriers, i.e. nitrate of soda and nitro-chalk, followed by barnyard manure, gave the highest yields of dry matter, protein and minerals.

5. This series of fertilizer treatments on old pasture showed what yields of dry matter, protein and minerals are to be expected from a predominantly bluegrass sward under the soil and climatic conditions of the experiment, which is fairly representative of Ontario conditions.

All combinations and single treatments except calcium gave significantly higher yields over no treatment.

In no case would the yield of available pasturage be considered high, which shows the inherent low yielding capacity of bluegrass.

It is important to observe that over the same 3-year period a seeded mixture of higher yielding grasses, clover, and alfalfa on the same soil type yielded 7260 lb. of dry matter, 1331 lb. of protein, 67 lb. of calcium and 21 lb. of phosphorus per acre which is more than double the yield of the highest production obtained by fertilizing bluegrass, and further that the yield of the mixture exceeds that of the original untreated sward of old bluegrass pasture by over 4 times.

These data give clear evidence of the low yielding capacity of bluegrass, and the value of plowing, cropping and reseeding old swards to productive temporary pasture mixtures.

ACKNOWLEDGMENTS

The author wishes to thank the Chemistry Department of the Ontario Agricultural College for carrying out the chemical analyses, and the office, field staff and students of the Field Husbandry Department who co-operated in carrying out the detailed laboratory and field work.

FALSE STING—A VIRUS DISEASE OF APPLES[1]

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[Received for publication September 11, 1940]

During the summer of 1934 attention was drawn to a mal-formed condition of the fruit produced on a few apple trees. All the fruit on these trees exhibited a degree of deformity. The condition so resembled the injuries produced as a result of apple red bug, green apple bug, or mullein bug feeding punctures that the term "false sting" was accepted as most descriptive.

Recommended measures for the control of insects have failed to reduce the production of deformed fruit on the trees in question, whereas adjacent trees have continued to bear normal fruit. Owners of the trees under observation have confirmed the fact that affected trees have borne "sting" fruit regularly during their bearing years. On some trees there has been a small percentage of marketable fruit, but the majority have produced fruit of cull grade only.

The condition has been under observation in the varieties Gravenstein, Baldwin, Blenheim, Ben Davis, King, Northern Spy, and Tolman Sweet in

FIGURE 1. Gravenstein apples affected with "false sting."

[1]Contribution No. 636 from the Division of Botany and Plant Pathology, Science Service, Dominion Department of Agriculture, Ottawa, Canada.
[2] Pathologist-in-Charge.

Nova Scotia. The disease has a superficial resemblance to stoney pit of pears, although no characteristic foliage or bark symptoms have been observed on apple trees affected with "false sting".

Scions from an affected Baldwin tree were grafted on two young McIntosh trees in 1935. Some of the fruit produced on this McIntosh wood in 1938 and also in 1939 appeared to be mal-formed. During 1940 the Baldwin scions and McIntosh stock each produced apples. The fruit of both varieties was typical of the "false sting" condition and appeared identical to that produced on the original tree. Adjacent McIntosh, similarly sprayed, have continued to produce normal fruit throughout this period.

The symptoms were first observed between two and three weeks after full bloom. As the fruit subsequently develops it becomes still more deformed in appearance. Figure 1 shows fruit collected approximately five weeks after full bloom.

From the history of the trees under observation it would appear that the only control for this disease is the eradication of affected trees. Only a comparatively few trees have been found affected in Nova Scotia and, so far as is known, no scions have been taken from these trees for the commercial propagation of the varieties.

The condition here described as "false sting" of apples is apparently a virus disease transmitted by grafting.

A DIPTEROUS PARASITE (*MYOPA* SP.) OF THE HONEYBEE[1]

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[Received for publication October 18, 1940]

While collecting nectar from the honey sacs of bees foraging sweet clover blossoms at Ottawa on July 9, 1940, the writer discovered a larva within the abdomen of a worker. The larva exhibited wriggling movements on removal but soon became motionless. It was white in colour, measuring approximately 3 mm. ($\frac{1}{8}$ inch) in length, and being somewhat pear-shaped with its posterior end enlarged, the last segment of which formed two wide curved plates. With the exception of a slightly swollen abdomen no further external evidence of parasitism was shown by the bee. Neither was there any evidence of internal injury as the alimentary canal appeared normal. Examinations made on numerous crawling bees disclosed no similar parasites.

The larval specimen was identified by C. T. Greene of the Division of Insect Identification, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Washington, D.C., whose help is hereby acknowledged, as *Myopa* species of the family Conopidae.

W. J. Brown, Senior Entomologist, Division of Entomology, Department of Agriculture, Ottawa, could find no reference to members of this genus as being parasitic on honeybees. Brown states as follows: "I have examined all the indices of American Economic Entomology and of the Review of Applied Entomology and have found no references to *Myopa* as a parasite of any hymenopteron. It thus appears probable that your record of the genus as a parasite of the honeybee is new as far as North America is concerned".

A general description of the habits of the Conopidae family are reported in a paper by Seguy (1). The eggs are deposited in some cases directly on the body of the host while it is in flight. Upon hatching the young larva enters the body of the host by perforating the lateral abdominal membranes. When fully developed the larval parasite occupies the whole of the abdominal cavity. The pupa rests enclosed in its puparium within the dead body of the insect during the winter. In the spring the young adult emerges from its protective cases.

According to Seguy, larvae of the genus *Myopa* parasitize *Vespa vulgaris*, *Andrena ovina* and *carbonaria*, *Bombus*, *Eucera* and *Colletes*. In this European record no mention is made of any members of the genus *Myopa* having been found as a parasite of *Apis mellifica*.

1. Seguy, E. Etudes sur les mouches parasites. I. Conopides, Oestrides, et Calliphorines de l'Europe occidentale. Encyc. ent., Paris, Sér. A. 9, 240 pp., 6 pls., 250 figs. 1928.

[1] Contribution from the Bee Division, Experimental Farms Service, Dominion Department of Agriculture, Ottawa, Canada.

[2] Agricultural Assistant.

A NEW FORM OF SPRUCE SAWFLY IDENTIFIED BY MEANS OF ITS CYTOLOGY AND PARTHENOGENESIS[1]

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[Received for publication February 15, 1940]

I. GENERAL INTRODUCTION

In November, 1930, serious defoliation of more than 2,000 square miles of spruce forest was discovered in the interior of the Gaspé Peninsula, Province of Quebec, Canada. This area of heavy infestation has now increased by some 10,000 square miles, and the insect causing it is known to be distributed throughout more than 145,000 square miles of northeastern America. The adults were identified as *Diprion polytomum* Htg., a species previously unreported in America, though known in Europe for more than one hundred years. In view of the economic importance of spruce, the suppression of such a destructive insect is clearly an urgent problem, and from the outset the Division of Entomology, Science Service, Dominion Department of Agriculture, has studied the outbreak closely to determine its probable development and to obtain information concerning the progress of parasite introduction which was started in 1931. A detailed account of our knowledge of its bionomics has recently been published by Balch (1). It is therefore necessary to deal with this phase of the problem only briefly in order to present certain of the data which suggested the investigation.

Bionomics

The sawfly overwinters in a cocoon spun in the forest floor and is thereby protected from the lethal temperatures of the Canadian winter. In a varying percentage of the cocoons this period of dormancy terminates shortly after the snow has gone, the majority of the adults usually emerging within a period of 2 or 3 weeks. However, there are always many stragglers which emerge irregularly throughout almost the entire season.

Reproduction is entirely independent of the male. Slits into which the eggs are inserted are made in the spruce needle by means of the saw-like ovipositor of the female. The eggs hatch after approximately 10 days and the young larvae first feed on the cortex of the older needles. In later instars they consume the whole needle. Feeding ceases after the fifth instar, and shortly after moulting to the sixth, the larva drops to the ground, burrows into the moss or litter, and spins its cocoon.

The number of generations produced varies both within and between regions. In general as the season becomes longer in more southerly districts, the number of generations increases. Thus in central Gaspé one generation is the rule, while two are typical of central and southern New Brunswick, and further south there is evidence that three are produced.

There is considerable variation in diapause; the percentage failing to emerge after overwintering varies both from year to year and place to place, becoming rapidly smaller in more southerly regions. In the Gaspé the

[1] See Section XIII, page 303, for a list of the institutions co-operating in this project.

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average is close to 80%, in central New Brunswick about 30%, and in southern New England possibly considerably lower. There is however marked annual variation which is reflected in the size of the larval population and hence in the amount of damage done from year to year.

Preliminary results from controlled experiments show that temperature has a marked effect on the tendency to go into diapause, and both temperature and humidity are of prime importance in breaking it. These experiments also point to the existence of lines genetically distinct with regard to their tendency towards diapause. While the habit clearly lowers the reproductive rate of any line in which it is firmly established, it probably acts as a guard against sudden decimation by unfavourable climatic conditions.

Although parthenogenesis is obligatory and gives rise ordinarily to females (thelytoky), males occur in the field in the proportion of about one to every 1200 females (47 out of 55,680 recorded emergences). In the material reared at the Fredericton laboratory males occur occasionally along with females as the progeny of virgin females. The line with the greatest frequency (93 ♂♂ : 3,361 ♀♀, or 1 : 37) is the one with the lowest tendency to diapause; it was reared continuously for 21 generations, although some individuals have gone into diapause. Field data suggest a possibility that males are somewhat more frequent in New Brunswick than in the Gaspé, but they are too few to be considered significant. Certainly there is no significant difference in their proportions from year to year.

The mating instinct is rudimentary. From two attempts observed the resulting progeny were all females. Since females are the normal progeny of obligatory parthenogenetic forms, as are the progeny resulting from fertilized eggs of facultative forms, the production of daughters supplies no evidence of fertilization. It is probable that if fertilization were successful, triploids would result and, being more or less sterile, these would diminish the reproductive rate to an extent depending on the frequency with which mating occurs.

The adult female flies strongly, sometimes at a considerable altitude, when with the assistance of strong air currents it must often travel many miles. Such strong flight combined with obligatory parthenogenesis naturally endows the species with enormous powers of dispersal.

Origin and Identity

Although first identified in 1930, two adults had earlier been collected, one from near Ottawa, Ontario, in 1922, the other from Mount Washington, New Hampshire, in 1929. They later proved to be *D. polytomum* also, thus indicating that the species has been widely distributed for some years. Extensive surveys since carried out by entomologists and foresters throughout Canada have afforded a fairly accurate picture of the present distribution of this insect, from which it appears not to occur west of longitude 81°.

Studies of cocoon population and normal increase lead to the opinion that the sawfly has probably been present in Canada since before the beginning of the present century (Balch (1)). It is at once clear that it possesses many characteristics which render especially strong the possibility of intro-

duction, but where it came from and at what point it was introduced it is not possible to say. If it is indigenous its restriction to the eastern part of its possible range, which extends north of the Prairies westwards to British Columbia, is difficult to account for. On the other hand, such restriction, together with the observed rapidity of spread, is readily compatible with the theory of origin by introduction at a date sufficiently recent to preclude the possibility of full occupation of the total spruce range.

Perhaps the strongest evidence that it is an introduction is the virtual absence of attack by native parasites. From some 276,000 cocoons collected throughout its known range and reared especially for the purpose during the past seven years, only 54 or less than 0.02% contained native parasites. These consisted of 18 different species.

With the exception of *D. nipponica*, indigenous to Asia, and *D. simile* and *D. frutetorum*, apparently introduced into America from Europe, the genus Diprion is confined to Europe. Such a distribution increases the probability that *D. polytomum* is an exotic species, though it might of course be circumpolar in distribution. Since obligatory parthenogenesis is probably derived from the facultative type, the Canadian form most likely arose where a closely related bisexual form occurs; no such form is known in Canada.

Comparison of the Canadian and European forms

Although a morphological comparison of the two forms failed at first to show the existence of any consistent structural differences, a preliminary investigation of the European form by K. R. S. Morriss of the Farnham House Laboratory, Farnham Royal, England, early disclosed some important physiological differences.

The seasonal history in Europe closely parallels that of the Canadian form, there being a single generation in the higher regions of Czechoslovakia and the more northerly countries such as Sweden and Finland, two in most of Czechoslovakia, and three in southern areas such as Hungary. There is, however, a striking difference in the location of the cocoons. The first generation is spun almost exclusively in the spruce foliage; the last generation is spun in the lower branches or surrounding herbaceous growth. Morriss reports, however, that in the colder localities a small percentage spins, like the Canadian form, in the surface covering.

A second difference concerns diapause. Again according to Morriss this tendency is not strongly shown, being limited in central Europe to about 5% of the population. It is possible that diapause might be somewhat commoner, especially in regions which have not yet been thoroughly investigated, but it is evident that the frequency of diapause is similar to that with which cocoons are spun in the moss.

The outstanding difference between the two forms undoubtedly concerns their respective methods of parthenogenetic reproduction. In Europe, judging from unpublished figures obtained by the Farnham House Laboratory from Czechoslovakian collections, the males appear to be approximately as numerous as the females, though there is of course considerable variation among the different collections. No information is

available from mated females, but virgin females reared by Morriss produced only males. It is clear therefore that this form, unlike the Canadian, is facultatively parthenogenetic (arrhenotokous).

As a preliminary to settling the question of the existence of two distinct forms it became necessary to investigate the causes underlying the parthenogenetic differences shown by the European and Canadian forms and if possible to establish some means of identification. At the request of the Dominion Division of Entomology the comparative cytological investigation was initiated, later to be substantiated by comparative rearing experiments at the Farnham House Laboratory, England, where in view of the relative scarcity of spruce such work could be carried out with safety.

II. INTRODUCTION TO THE CYTOLOGY OF PARTHENOGENESIS

One of the main characteristics of the Hymenoptera is their ability to reproduce by means of parthenogenesis, whereby in different forms either males or females are produced without recourse to mating. According to Vandel (53), Dzierzon (16) in 1845 was the first worker to give a satisfactory explanation for the peculiarities of reproduction in the domestic bee. He was of the opinion that all the eggs of the bee are similar and that at the moment when they pass in front of the *receptaculum seminis* they can be fertilized or not. If the eggs are not fertilized they will give rise exclusively to males; if they are fertilized they will give rise to zygogenetic, that is, biparental, females. Therefore in this type of parthenogenesis, variously known as facultative, arrhenotokous, or haploid parthenogenesis, the determination of sex is directly related to the success or failure of fertilization.

It was not until the work of Meves (30) in 1907 that Dzierzon's theory received cytological support. Working on the domestic bee also, he showed that the first division of spermatogenesis was completely abortive, and that the sperm therefore carry the number of chromosomes characteristic of the somatic nuclei. This is striking confirmation, for since fertilization consists essentially of the union of two haploid nuclei the development of an egg without first being fertilized (though capable of being fertilized) should result in an individual with a number of chromosomes half that of its mother. Since that time comparable conditions have been reported in all the facultative species examined (cf. Sanderson (38) for a detailed treatment). The observed absence of fertilization and the abortive nature of spermatogenesis are thus regarded as conclusive evidence that facultative males are haploid in constitution. But females also can arise from unfertilized eggs and the discovery of Torvik-Greb (51) and of the present author that diploid spermatocytes likewise undergo an abortive spermatogenesis demonstrates that at most hymenopteran males are haploid only relative to the female.

Although on occasions females develop normally from unfertilized eggs, that is, by obligatory, thelytokous, or diploid parthenogenesis, so far as is known they are never haploid (F. and S. H. Schrader (39)). It is clear therefore that some modification of the meiotic process is necessary in order to suppress, or compensate for, the customary reduction. Unfor-

tunately only a very limited number of obligatory species has been investigated, and the work is not sufficiently critical in all cases to remove doubt concerning the cytological conditions obtaining.

The various ways in which meiosis can be suppressed may be grouped into two main classes depending on whether synapsis is complete or incomplete. According to Darlington (12, p. 450) there are four modifications of meiosis following either partial or complete asynapsis (two are confined to the plant kingdom and will therefore be omitted from consideration here) and three following complete synapsis. Recently two new modifications have been reported, both of which follow apparently normal chromosome pairing, but as will be shown their interpretation meets with certain objections both from the cytological and the genetical points of view.

Where there is no attempt at pairing (Table 16, Type I) the diploid number of univalents may divide once to give a single polar body and the female pronucleus (*Thrinax macula*, Peacock and Sanderson, (33)), or twice (Type II) to give two polar bodies (*Cocconeis placentula*, Geitler (19)).

Pairing is complete in the second class, and the haploid number of bivalents appears on the first metaphase plate, but their subsequent behaviour is varied: first, the derivatives of the second anaphase may fail to separate (Type V, *Rhabditis monhystera*, Belar (3)); second, the two groups of chromosomes may reunite at second telophase (Type VI, *Lecanium hesperidum*, Thomsen (48)); and third, the daughter nuclei of an early cleavage division may reunite (Type VII, *Trialeurodes vaporariorum*, Thomsen, (48)).

Recently Dodds (14) has shown in the female producing generation of *Neuroterus baccarum* that the metaphase spindle is formed not perpendicular to but parallel to the margin of the egg, and that "more than 10 (*the haploid number*) chromosomes were proceeding to each pole .... Two nuclei, both of which later undergo segmentation divisions, are reconstituted at the poles." In the initial stages of segmentation the diploid number of chromosomes was consequently seen. This is Type III in Table 16. A further modification of meiosis (Type IV) was reported by Speicher (45) for *Nemeritis canescens*. Here again synapsis is apparently normal, but the bivalents this time are arranged on the spindle perpendicular to the periphery of the egg. At the first division the two groups of dyads fail to separate, but instead they remain together as if to form a restitution nucleus. The second division, however, is remarkable in not being equational or mitotic in nature, but in consisting of the separation of one haploid set of dyads from a second set. Presumably the constituents of each dyad subsequently dissociate, for at the ensuing cleavage the diploid number of chromosomes is seen. It is evident therefore that some special mechanism must be invoked to ensure the segregation of one complete haploid set of dyads to each pole; the nature of this mechanism is not yet understood.

III. CYTOLOGICAL METHODS

The large majority of somatic preparations were made from the neural cells of pronymphs, that is, during metamorphosis from the larval to the adult brain. The head-capsule was cut open, a few drops of fixative injected, and the whole brain dissected out and fixed in a modification of

Kahle's (24) fluid (15 parts 95% alcohol + 6 parts commercial formalin + 1 part glacial acetic acid). After 5 minutes fixation the material was transferred to a drop of 45% acetic acid on a slide which had been previously smeared with a fine film of egg-albumen and had been heated until dry. A cover was then placed over the material which, after being flattened with moderate pressure, was heated 3 or 4 times over an alcohol flame. The cover was floated or pried off in 70% alcohol and the slide stored until ready for staining.

Staining was done by means of Feulgen's "Nuklealfärbung" method (Feulgen and Rossenbeck (18)). The material received 14 to 16 minutes hydrolysis and was stained for $1\frac{1}{2}$ to 2 hours. On counterstaining with light green in 70% alcohol the red colouration of the chromosomes changes to violet and the cytoplasm takes on a contrasting green colour. With the appropriate amount of pressure the chromosomes are both flattened and spread, thereby greatly facilitating the study of their morphology.

By the same method additional counts were obtained from the developing wingbuds of pronymphs just prior to evagination, and from the enlarging ovariole walls of pronymphs and pupae. Similarly the ovaries and testes of pronymphs supplied excellent preparations of oogonial and spermatogonial chromosomes.

Oogenesis was studied mainly in embedded and sectioned material, although the earlier stages of prophase were superior in preparations made as described above; the testes were almost invariably treated by the smear method (cf. La Cour (26)). As a rule the ovaries were dissected out in a drop of the fluid used for fixation, but occasionally with ovaries and always with testes a physiological salt solution was injected into the exposed viscera in such a way that the majority of the enveloping fat bodies floated away. Either frog Ringer or a 0.75% NaCl solution was used.

Oogonia and early oocytes were present in the early pronymphal stage, but this was too early for spermatogenesis; consequently males were not used until they had reached the pupal stage and had become fairly darkly coloured.

A large number of standard fixatives was tried; for testes smears La Cour's 2BD (25) proved best, while for ovaries the best figures were obtained with modified Kahle's fluid. Before haematoxylin a second modification of Kahle's fluid was not infrequently used; it consists simply in the replacement of the water normally present by a corresponding amount of a saturated solution of picric acid (this is referred to as "Picro-Kahle").

The final stages of maturation were found to be undergone within 45 minutes after oviposition. The seriation of the stages was determined by noting the time at which the female had finished oviposition, allowing the egg to incubate for the appropriate time, and then fixing in modified Kahle.

The fixed material, apart from smears, was almost invariably dehydrated by Zirkle's (63) N-butyl alcohol method, but the constituent steps were shortened so that the material was ready for sectioning within $2\frac{1}{2}$ days after dissection.

Ovaries were sectioned at from 6 to 16 microns; the thinner sections provided excellent somatic and oogonial counts, while the thicker ones were more appropriate for the study of late prophase in the relatively large primary oocytes. Great difficulty was experienced in sectioning the incubating eggs, due to the hard chorion, the brittle yolk, and to the toughness of the spruce needles in which they are laid. To circumvent these difficulties a new procedure was adopted which combined the most favourable features of three other techniques (Zirkle (63); Petrunkevitch (34), and Slifer and King (40)). After the required incubation period the needle is removed from the branch, the egg partly exposed by enlarging the nidal slit, and the whole plunged into modified Kahle. After 2 or more hours fixation the egg is dissected out of the needle and treated according to the schedule of Smith (43).

Heidenhain's iron haematoxylin gave excellent but variable results; Moreover, the prolonged differentiation necessary for the optimum staining of the chromosomes entails the removal of much of the stain from the centrosomes and spindle fibres. Newton's crystal violet staining (cf. Huskins, (23)) was practised to only a limited extent, but the chromic acid modification of Belar (cf. La Cour, (26)) proved invaluable in the study of the maturation stages in the laid eggs. In an endeavour to trace the history of the chromatin through the growth phase of the oocytes, where the staining of the meiotic chromosomes is especially difficult, Feulgen's "Nuklealfärbung" method was tried in addition to haematoxylin and crystal violet. It gave no reaction after pachytene.

A summary of the various stains and fixatives used in this study along with the various tissues from which material was obtained are given in Table I.

All drawings were made with the aid of an Abbe camera lucida, using either a 1.5 mm., 1.3 N.A. Zeiss apochromatic objective, or a 1/12 Zeiss Homogene immersion objective with a 20 or a 30× ocular. At bench level with these oculars the former objective gives magnifications of 4400 and 6600 diameters, while the latter gives 3600 and 4500 diameters.

TABLE 1.—SUMMARY OF CYTOLOGICAL TECHNIQUE

Type of cell	Developmental stage	Tissue	Fixative	Stain
Somatic ♂ & ♀	Pronymph	Brain and wing-buds	M.K.	Feulgen
Somatic ♀	Pronymph and pupa	Ovariole wall	M.K. and A-A.	Feulgen
Spermatogonia	Pronymph	Testes	M.K. and A-A.	Feulgen
Spermatocytes	Pupa	Testes	2BD and Nav.	Feulgen
Oogonia	Pronymph	Ovaries	M.K. and A-A.	Feulgen
Early oocytes	Pronymph and pupa	Ovaries	M.K. and A-A.	Haem. and C.V.
Late oocytes	Laid eggs 10 to 45 mins. incubation		M.K.	Chromic C.V.
Somatic	Embryo after 45 mins. incubation		M.K.	Feulgen

M.K. = modified Kahle's fluid; A-A = absolute alcohol : acetic acid : : 3 : 1; Nav. = Navaschin's fluid; Haem. = Heidenhain's iron haematoxylin; C.V. = Crystal violet; Chromic C.V. = chromic crystal violet

IV. CYTOLOGICAL OBSERVATION

Material

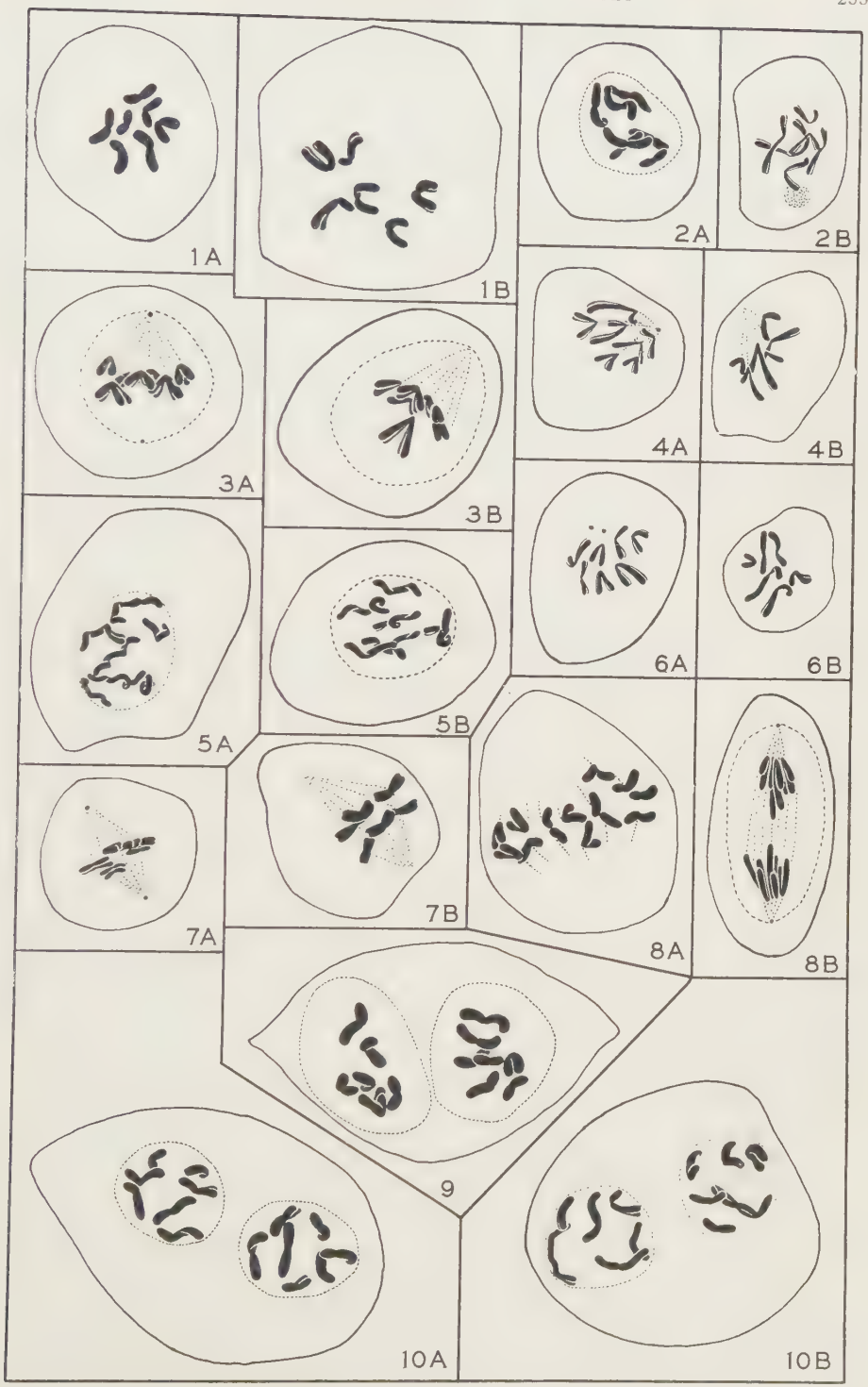
For the preliminary comparative study of the Canadian and European forms all the males and the majority of the females of the former came from the 21-generation line raised at the Dominion Entomological Laboratory, Fredericton, N.B., from material collected locally. Additional females came from cocoons collected at Parke Reserve, Cascapedia and Causapscal, typical 1-generation areas in the Gaspé Peninsula, while others were obtained from Young's Brook and Tay Creek, 2-generation areas in the immediate vicinity of Fredericton. Regardless of the place of origin no cytological differences could be detected.

The European material was obtained from cocoons collected as larvae in the summer of 1935 at Hradec u Opava, C.S.R. These had been allowed to spin cocoons, and were subsequently stored in the cold chamber at the Dominion Parasite Laboratory, Belleville. Between July 21 and August 12, 1936, the total emergence figures were 1257 males and 3455 females. A certain undetermined number had emerged prior to shipping from the Farnham House Laboratory, England, so that these figures cannot be used to establish the sex ratio: nevertheless the form is undoubtedly facultative.

Spermatogenesis

Spermatogonial mitoses show 7 chromosomes in the Canadian form and 6 in the European form (Figures 1A and 1B). In the primary spermatocytes the chromosomes are at first long and drawn out, then gradually shortening, but without undergoing a process of synapsis they come to occupy the centre of the nuclear space (Figures 2A and 2B). Though from the number of separate members present there has clearly been no pairing of the chromosomes, each is seen to be double throughout except for the region near the centromere (Figure 2B). The nuclear membrane probably never breaks down. Close to it lies the centrosome from which radiates a complete half-spindle to the chromosomes arranged loosely on the equator (Figures 3A and 3B). The ensuing anaphase involves no division of the nuclear mass; all the chromosomes migrate to the centrosome, and as they do so the double nature of each is especially clear (Figures 4A and 4B). At the pole they enter into a short telophase (Figures 5A and 5B) but never lose their individuality. No cytoplasmic bud is pinched off at the opposite pole, though these representatives of a secondary spermatocyte have been observed in some Hymenoptera.

As the chromosomes arrange themselves on the equator (Figures 6A and 6B) in preparation for the second division, the spindle is seen to be complete, being composed of 2 centrosomes with a half-spindle running from each to the chromosomes (Figures 7A and 7B). The number of chromosomes is here again clearly 6 and 7 for the European and Canadian forms respectively, proving that there has been no reduction in number since the spermatogonial divisions. The nuclear membrane remains intact. As the half-chromosomes separate along the longitudinal split (Figure 8A) and move to the opposite poles (Figure 8B), the cell becomes elliptical in form and the daughter chromosomes enter the second telophase (Figures 9A,



FIGURES 1A to 10B. Spermatogenesis in the Canadian male (A) and the European male (B): 1, spermatogonial mitosis; 2, metaphase in primary spermatocytes—polar view; 3, ditto—side view; 4, anaphase in primary spermatocytes; 5, first telophase; 6, metaphase in secondary spermatocytes—polar view; 7, ditto—side view; 8, early and late anaphase in secondary spermatocytes; 9 and 10, second telophase.

10A and 10B). At this stage the number of chromosomes is still readily demonstrable and, since the cell cleaves to form the two daughter nuclei which will each give rise directly to a sperm, there can be no doubt that the spermatozoa of the two forms differ in the number of chromosomes they carry, and further that these are the same as their respective spermatogonial numbers. This identical number is thus directly referable to the absence of a reduction at the first division of meiosis.

Oogenesis

At the beginning of the pronymphal stage each ovariole contains 2 or 3 well-formed oocytes, the largest of which is the most posterior, i.e., is situated nearest the oviduct. At the anterior end of the ovariole is a patch of epithelial cells within which is the zone of actively dividing oogonia aggregated into rosettes. In the earliest stages seen the rosettes consist of 4 cells, which later divide to give 8, then 16, and finally 32 derivatives which later are differentiated into the nurse cells and the single oocyte. The oocyte is the most posterior cell in each rosette and becomes incompletely separated by a follicular layer from the remaining cells which constitute the nurse chamber. The latter are at first very prominent and alternate with the much smaller oocytes, but with the elongation, separation and increase in girth of the ovarioles, a reversal in their relative sizes occurs owing to the growth of the oocytes at the expense of the nurse chambers (Figure 11).

In the oogonia, metaphase plates show that the Canadian and European females possess 14 and 12 chromosomes respectively (Figures 12A and 12B). After the final oogonial division in which the daughter cells destined to produce primary oocytes and nurse cells retain the diploid number of chromosomes, the nuclear behaviour is not eadily determined. Following the last premeiotic anaphase the chromosomes are next seen as somewhat loosely spiralled elements present in the diploid number (Figure 13A), but it is uncertain whether a true resting stage follows or whether the dense lateral clump which is formed results from the collapse of the leptotene chromosomes. That the latter is more probable is suggested by the apparent absence of a characteristic leptotene; no leptotene is figured by Sanderson (38) in *Pteronidea ribesii*, nor by Speicher (45) in *Nemeritis canescens*.

As the cell increases in volume the dense lateral clump resolves itself into a number of relatively thick loops of greater or lesser length radiating out from a point of polarization on the nuclear membrane. This is the well-known bouquet stage regarded by Gelei (20) as an adaptation to ensure regularity in pairing. When the number of threads can be counted it is found that there are 6 in the European (Figure 83) and 7 in the Canadian (Figure 82). Since these are the haploid numbers it is clear that though the threads are usually optically single, they must in reality be double as the result of the close association of homologous chromosomes in pairs. That this interpretation is correct and that the association is lateral in nature is shown by the rare occurrence of nuclei at zygotene (Figure 14A) and cases of incomplete pairing where interstitial and terminal regions

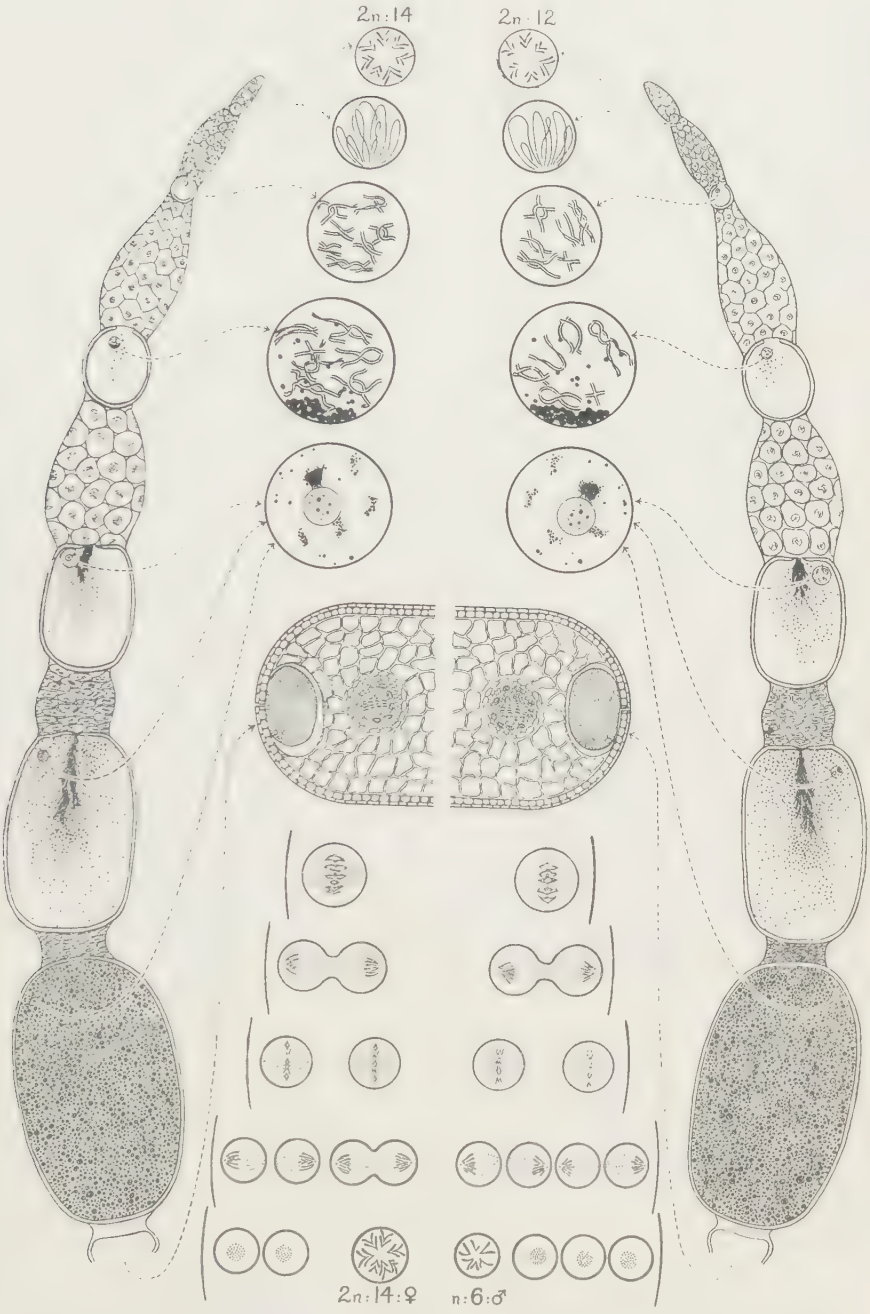


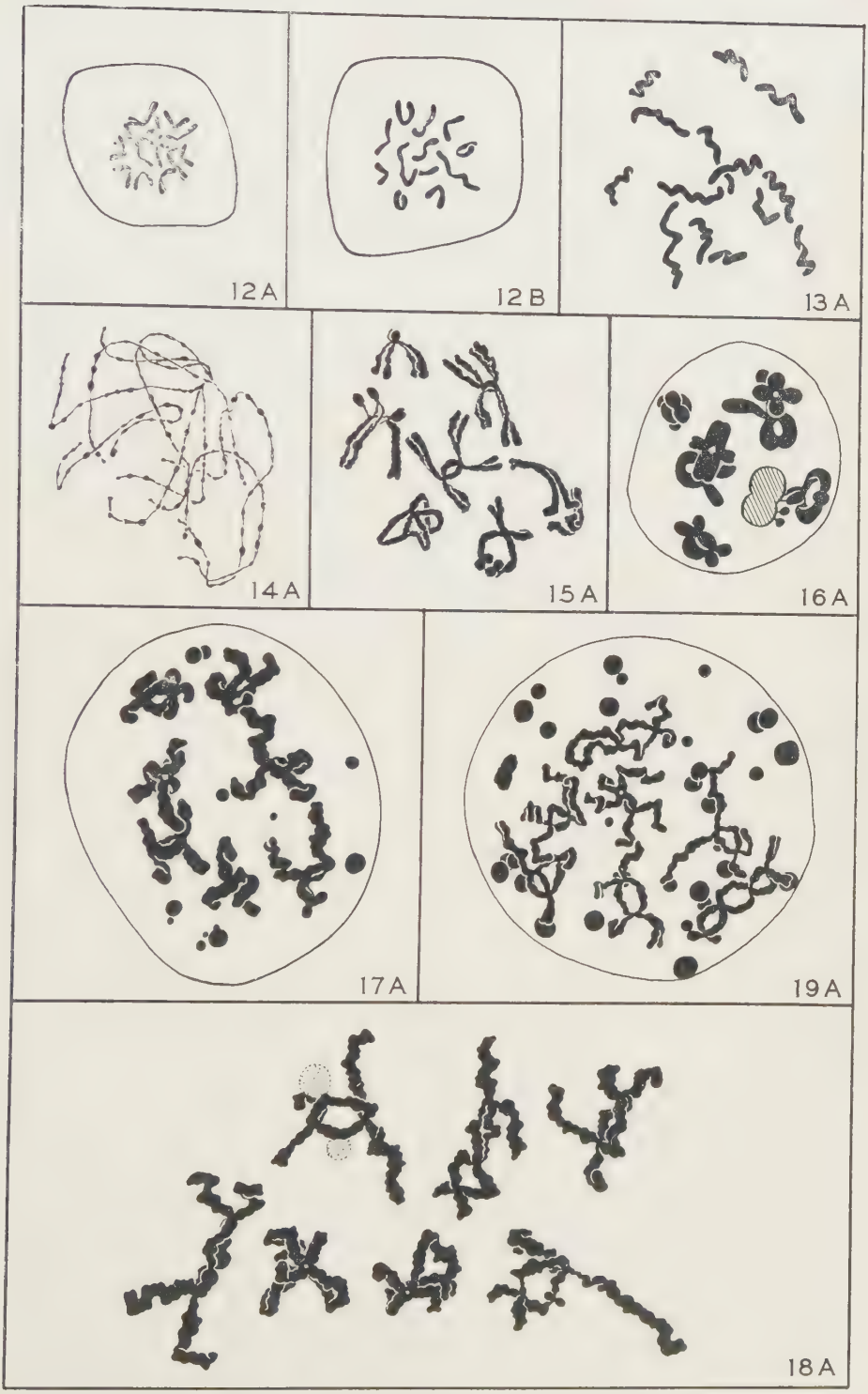
FIGURE 11. Diagrammatic comparison of meiosis in the Canadian (obligatory) and European (facultative) females. To the left and right are represented longitudinal sections through their respective ovarioles (consisting of alternating primary oocytes and nurse chambers). At the upper centre the oogonia and oocytes are enlarged. Below and in order are cross sections of half-needles containing the eggs and the successive stages of maturation (assumed for the facultative form) culminating in the production of diploid eggs by the obligatory form and haploid eggs by the facultative form.

are clearly double (Figure 75). The rarity of cells with incomplete pairing indicates that the process is of short duration and is restricted to the early stages before the cell expands. The chromosomes of all the nuclei within each rosette show this paired condition, and since only one cell becomes the oocyte, the others serving a nutritive function as nurse cells, it follows that the latter are aborted eggs.

As the visually single pachytene threads lose their polarization their dual nature is shown by the constituents of each pair falling apart into loops held together by the crossing threads (chiasmata). That these bivalents are now four-partite can be seen on close inspection (Figure 15A). From this point on the behaviour of the oocyte and its associated nurse cells is quite different. The bivalents of the nurse cells disperse throughout the nucleus, lose their definition, and appear to increase in size by becoming granular. Meanwhile the cell boundaries gradually disappear and the nuclear granules lie free in the cytoplasm of the nurse chamber, later to be passed with it into the ooplasm. The rapid elongation of the ovarioles which is occurring at this time is due mainly to the great increase in size of the nurse cells, for the oocytes remain relatively small and wedged in between 2 adjacent nurse chambers. In general, 5 or 6 oocytes are differentiated in each ovariole and, since they are formed progressively, the sequence of events during prophase can be followed throughout.

In the developing oocyte the germinal vesicle is at first proportionally small, but as the oocyte increases in bulk the size relationship is maintained so that finally the nuclear volume is considerably greater than when the oocyte was first differentiated. In pollen mother-cells of plants and during spermatogenesis in this insect there is a progressive condensation due to spiralization of the chromosomes from pachytene to metaphase. But in the oocytes of *Pristiurus* (Rückert, (37)) and *Scolopendra* (Bouin, (5)), perhaps due to the maintenance of a constant volumetric relationship between the chromatin and the cytoplasm, there is an increase in the absolute size of the chromosomes. The same conditions were noted in the present material up to well-advanced diakinesis but whether this represents a decrease in size when measured in relation to the increasing volume of the cytoplasm has not been determined. Further, so long as the volume of the nucleus is small the bivalents are found to be evenly arranged against the nuclear membrane (Figure 16A) suggesting the existence of a strong surface charge on them (Lillie, (27)) which is manifested in the form of a repulsion. But when the cell has reached its maximum size the chromosomes are no longer evenly distributed but are sometimes situated at one side of the nucleus (Figure 23B); presumably due to a disproportionate increase in the distance between the chromosomes and the charge on them the repulsions are now inadequate.

After pachytene the chromosomes fail to stain with Feulgen's stain, but continue to react to Heidenhain's haematoxylin until late diakinesis after which it has proved impossible to locate them with any stain until first metaphase. In Figures 17a, 18a, and 19a, the 7 bivalents of a Canadian female pronymph are shown at mid diakinesis and in Figure 15a is shown the complement of a younger oocyte. The 6 bivalents in oocytes of the European form are shown in Figures 20B to 23B.



FIGURES 12A to 19A. Oogenesis in the Canadian obligatory female (A) and the European facultative female (B): 12, oogonial mitosis; 13, premeiotic telophase (?); 14, zygotene; 15, early diplotene; 16, the 7 bivalents of a nurse cell—note repulsion; 17 to 19A, mid diakinesis—the 7 bivalents in Figure 18 are drawn separately.

Since a chiasma represents an exchange of partner among four homologous chromatids associated in pairs, it is, in general, responsible for the maintenance of the paired condition and thus is a prerequisite to an orderly reduction of the chromosomes to one-half of the diploid number. Moreover since chiasmata are formed in the oocytes of both the facultative and the obligatory forms, it follows that the early conditions in the eggs of both are such as should ensure that the eggs will ultimately undergo a process of reduction and come to possess the haploid number of chromosomes.

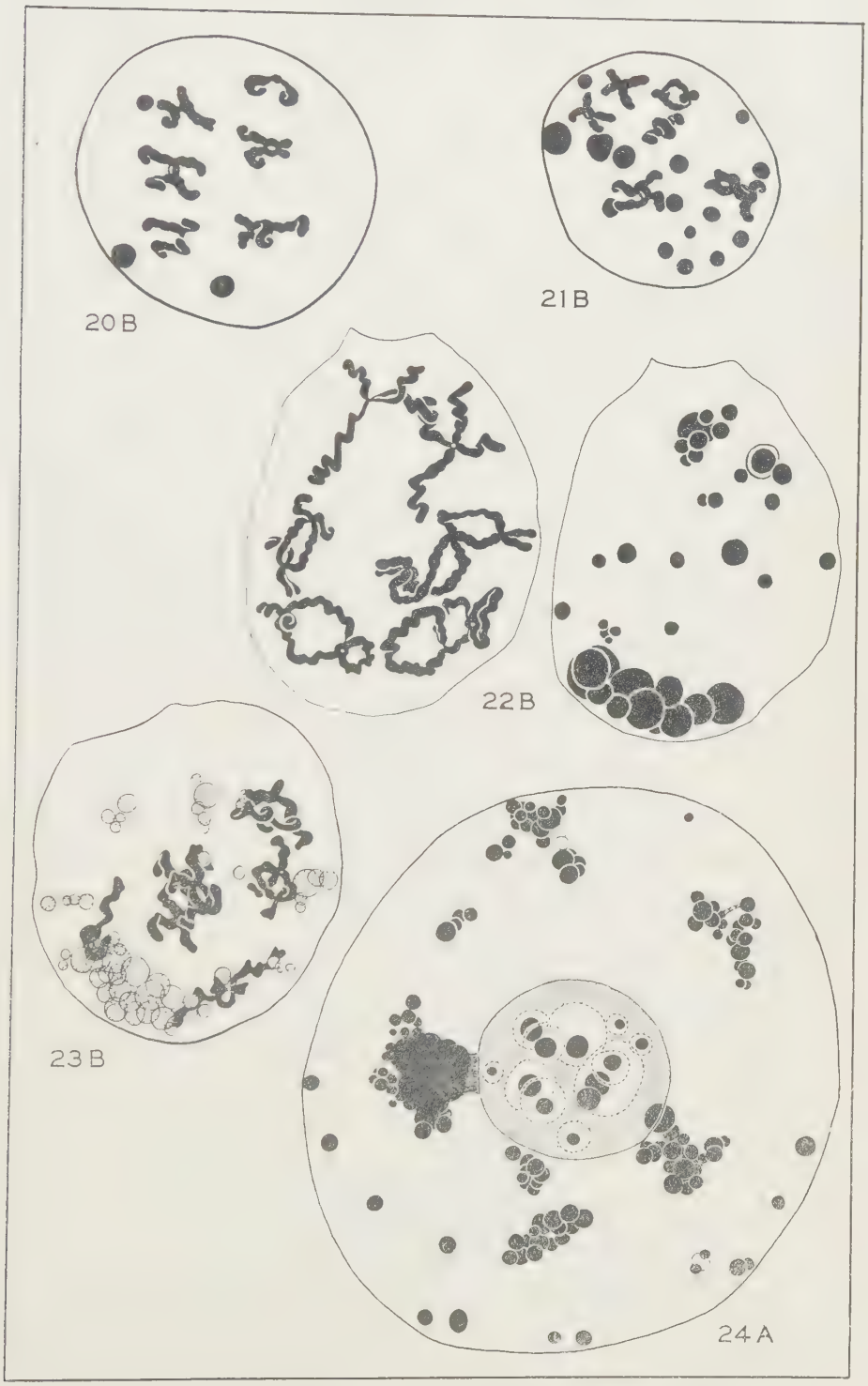
As is general in the Hymenoptera great difficulty has been experienced in establishing the history of the bivalents after mid diakinesis. It is certain that subsequent to this stage the chromosomes contract to a high degree and reach a condition where a "normal" reaction to haematoxylin, aceto-carmine, crystal violet and Feulgen does not obtain. The decrease in size occurs coincidentally with the loss from the chromosomes of an extra-chromatic material which resembles nucleolar substance in its staining reaction and which is visible as globules being budded-off from the bivalents from mid diakinesis on. The globules accumulate at one side of the nucleus against the nuclear membrane (Figures 22B and 23B) through which they appear to pass out into the surrounding yolk (Figure 11). Finally, there remains no sign of typical bivalent chromosomes, but a composite body is present (Figure 24A) the nature of which is not yet understood.

In both forms the maturation divisions are not undergone until after the egg is laid, this act presumably being the stimulus to renewed activity on the part of the nucleus. The following description refers only to the eggs of the Canadian form, as those of the European form were not available. The latter, however, in view of their facultative nature, would be expected to undergo a normal reduction as has been demonstrated for other facultative species (cf. Sanderson, (38)).

Maturation of the Egg

The mature egg, invested by a thin but tough chorion, is laid longitudinally in a slit in the needle and, as estimated from embedded and sectioned material, measures about 250 × 600 × 1800 microns. Since sectioning is more successful in the transverse direction, there result at 14 microns some 130 sections. The egg is not completely symmetrical, being slightly rounder at the anterior end about one-quarter of the way from which the nucleus is situated, so that its approximate position can be estimated. At the first metaphase of meiosis the nucleus, now devoid of a nuclear membrane, lies in a yolk-free patch of cytoplasm close to the periphery of the egg (Figure 25). The equatorial plate is formed more or less parallel to the boundary of the egg and the chromosomes are arranged on it as 7 bivalents, minute in comparison with their size at mid diakinesis.

On account of the close proximity of the egg boundary, anaphase disjunction is accomplished, not by a mutual separation of the constituents of the bivalents, but by a movement of the inner 7 away from their partners left almost stationary on the equator. Such a demobilizing effect has been shown by Darlington in the case of pollen grain walls in *Tradescantia* (7) and *Podophyllum* (11). The position of the two groups of chromosomes



FIGURES 20B to 24A. Oogenesis in the Canadian obligatory female (A) and the European facultative female (B): 20 to 23, mid diakinesis—in Figure 22 the extrachromatic globules are illustrated separately from the chromosomes; 24, the composite body in an oocyte prior to oviposition.

relative to the egg periphery at mid anaphase (Figure 26) and at late anaphase (Figure 27) shows that separation is due mainly to the movement of only one group; moreover, the inner group alone is seen in the characteristic attitude of movement. Often the spindle is not perpendicular to the margin of the egg but is directed at an angle towards the centre of the egg. This stage is followed by a telophase of short duration (Figure 28).

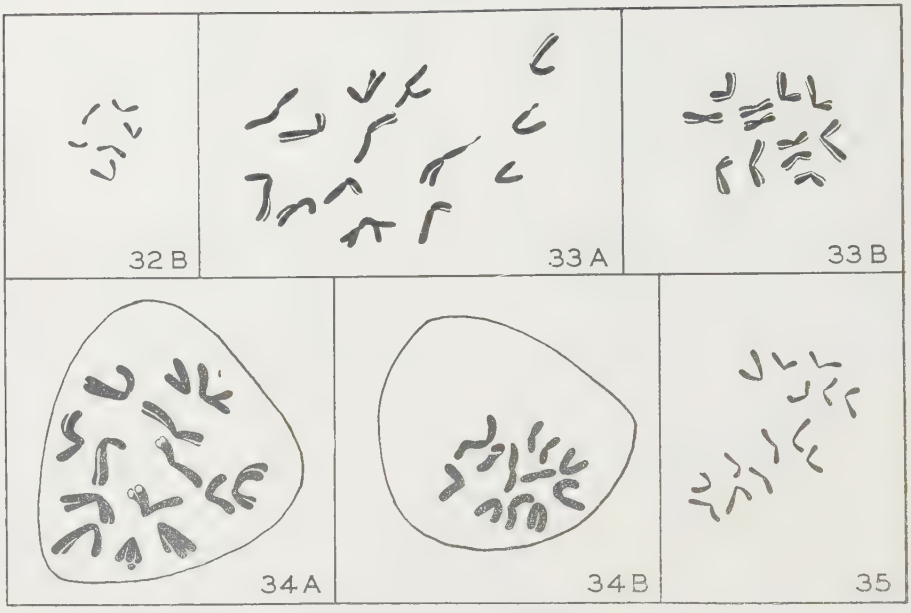
The second division spindles are contiguous and approximately in line with that of the first division. Seven chromosomes line up on each equatorial plate (Figure 29) and their chromatids separate at second anaphase to form 4 nuclei. In 3 of the late anaphase nuclei so formed 7 chromosomes could readily be counted, but in the innermost it was not possible to determine the number directly as the chromosomes were grouped too closely (Figure 30). In other eggs of approximately the same age only 3 nuclei are present while in older eggs a lone nucleus is present which shows the diploid number of chromosomes, 14 (Figure 31). It is therefore inferred that the female pronucleus and the second polar body have re-fused to re-establish the diploid chromosome number. It is unlikely that the 14 chromosomes could be the result of the fusion of the derivatives of the first cleavage division: first, because of the brief incubation period, and second, because of the sub-marginal position of the nucleus—in related forms cleavage starts only after the egg nucleus has sunk into the centre of the yolk mass.

Somatic Chromosomes

The study of the somatic chromosomes has been restricted to the nuclei of pronymphs and pupae, both male and female in the case of European material, but only female in the case of Canadian material, the rare males being used solely for the study of spermatogenesis.

By far the best figures were obtained from the neural ganglia which showed 6 and 12 chromosomes for the European male and female and 14 for the Canadian female (Figures 32B, 33A, and 33B). Identical counts were obtained from the developing wing buds and the ovariole walls (Figures 34A and 34B), but numerous polyploid nuclei were encountered in all somatic tissues. In wing buds almost all the nuclei had double the number seen in the germinal tissue; in the ovariole walls octoploid cells were not infrequently observed; while nuclei in the brain were occasionally seen with higher numbers of chromosomes. The occurrence of polyploidy in the cells of certain somatic tissues is a phenomenon well-recognized in the Insecta (Wilson, (62, p. 870)). The condition is not specific for all nuclei of the individual, being more common in cells which are old, highly specialized or degenerating. In the present material polyploidy is found only where a rapid increase in number of cells is necessary either to keep pace with the greatly increasing size of the ovaries and the rapid development of the wings, or because of the short period of time available for the metamorphosis of the brain. It is significant that no instances of polyploidy were observed in the germinal cells.

FIGURES 25 to 31. The maturation divisions in eggs laid by Canadian females. In order the stages are: metaphase I, early anaphase I, late anaphase I, telophase I, metaphase II, anaphase II, and the fusion nucleus containing 14 chromosomes.



FIGURES 32B to 35. The somatic chromosomes of Canadian (A) and European (B) individuals: 32, the neural chromosomes of a facultative male; 33, ditto in females; 34, the ovariole wall chromosomes; 35, the 14 neural chromosomes of a female from Albrechtice, Silesia.

A consideration of the chromosomal and physiological differences (see Table 2) suggests that two distinct forms are involved. However, it does

TABLE 2.—SUMMARY OF DIFFERENCES

	European	Canadian
Parthenogenesis	Facultative	Obligatory
Chromosome number ♂	6	7
♀	12	14
Cocoons in moss, %	5	*ca* 100
Diapause, %	5	30–80

not eliminate the possibility of the two forms occurring together in Europe, for if they overlap in distribution and their progeny mix, this would mask the fact that in one of them parthenogenesis is obligatory and leads to the production of females only. Unless this is so the differences observed must have been acquired since introduction into Canada. On the other hand, the introduction into a highly suitable environment almost free from native parasites, of a species which due to its purely parthenogenetic nature has a high reproductive rate and great powers of dispersal, and a habit of spinning its cocoons in protected locations, meets all the requirements demanded of the theory that it was introduced in its present form.

The identification of a single female with 14 chromosomes (Figure 35) in a collection of *D. polytomum* from Albrechtice, Silesia, strengthened the possibility that two chromosomally distinct forms of this species occur in Europe. It was therefore deemed advisable to undertake the breeding of European females obtained from widely scattered regions in order to test the possibility of the existence there of two parthenogenetically distinct forms, and by means of the progeny to correlate parthenogenetic type with chromosome number. This was carried out with the aid of a Royal Society of Canada Fellowship at the Farnham House Laboratory and at University College, London, England.

V. COMPARATIVE BREEDING EXPERIMENTS

Material

Collections have been tested from 15 regions in Czechoslovakia, and pertinent data regarding them are given in Table 3. It will be noted that two collections were made from Jenikov, Moravia, but since they were secured at different altitudes they have been treated separately throughout.

TABLE 3

Locality	Height in metres above sea level	Generations per year
Spindelmuhle, Riesengebirge Mts., North Bohemia	600 – 700	2
Klenec, North Bohemia	500 – 600	2
Dobris, Brdy Wald, Mid Bohemia	400	2
Hvozdany, Brdy Wald, Mid Bohemia	600	2
Myto, Mid Bohemia	300 – 400	2
Kout, West Bohemia	400 – 500	2
Klatovy, West Bohemia	500	2
Stepanov, East Bohemia	350 – 400	2
Kunzak, South Bohemia	600 – 700	2
Plese, South Bohemia	500	2
Albrechtice, Silesia, North Moravia	600 – 700	2
Jenikov, West Moravia	600	2
Jenikov, West Moravia	400 – 500	2
Ruzomberok, North Slovakia	700 – 800	1
Brezno, Lower Tatra Mts., Mid Solvakia	900	1

Small samples of cocoons were removed from cold storage as required and kept in a breeding room maintained at a temperature of 75 to 78° F. The relative humidity in the room was recorded continuously throughout the experiments, but it has not been taken into account as it proved variable and in the actual rearing cylinders it was considerably higher.

After a period of 5 to 7 days, or even longer, the adults emerged. At first, if both males and females were available at the same time, one of each was set up in a cylinder in an attempt to secure mating. However, when it became obvious that not only was there no desire to mate, but that the presence of the male was having a disturbing effect on the female's attempts to oviposit, this practice was discontinued. Numerous and varied efforts were made to secure matings either between males and females of

unknown constitution, or between miscellaneous males and females of known parthenogenetic type, or finally, on a few occasions, between individuals clearly belonging to a particular race. In no case were these efforts successful. Moreover, the interference resulting from the presence of the male could in some cases be held responsible for the low number of progeny resulting.

Soon after eclosion, females were placed on second year foliage of *Picea excelsa* enclosed in a glass cylinder 12″ in length and 4″ in diameter. The cut end of the stem was submerged in a vial of water and held in position there by a plug of non-absorbent cotton-wool. In the cylinders the vials stood on damp blotting-paper contained in flat trays, the upper ends of the cylinders being closed by means of cheese cloth. By moistening the blotting-paper three times a day the relative humidity in the cylinders was kept considerably higher than that of the room as a whole.

Whenever possible a detailed record was kept of the time of emergence of the adult; the approximate times at which the first and last eggs were laid; the number of larvae that hatched, together with the time at which the first appeared; the general colour and pattern of the various instars; the date of the final (fifth instar) moult; the date at which the first and last cocoons were spun; and the total number of progeny spun in the foliage as opposed to the number spun in the Sphagnum mass which was supplied during the fifth instar as a layer on the bottom of the cylinder. Finally, the moulted fifth instar head-capsules were retained in the case of female progeny, and the length of one larva measured as accurately as possible. After some 10 to 12 weeks in cold storage the individual cocoons of the various female progeny were weighed in order to determine their relative variability in gross size both within and between families. From these data an attempt has been made to determine (necessarily rather roughly) whether there are significant differences between the various families reared.

For comparative purposes three rearings were made of the Canadian form from material originating from Young's Brook, N.B. Occurring freely in four of the Czechoslovakian collection were cocoons of another species, *Diprion abieticolor*, D.T., which, however, could be distinguished from *D. polytomum* by the colour of the larve and pupae and the general morphology of the adult. Five families were reared from this species. Finally, a single rearing was made from a third species, *D. nemorum*, F. von Gmel.

Observations

D. polytomum—Czechoslovakia

A total of 50 first generation families was raised from material coming from 15 different regions in Czechoslovakia, and from 3 of these familes 7 second generation families were reared. The single female tested from Ruzomberok produced only 1 progeny, a male, and therefore appeared to be a facultative form, but as will be shown later there are morphological grounds for believing it to be obligatory. Because of the uncertainty regarding its reproductive category it will be omitted from consideration until later (p. 282). As will be seen from the summarized results in Table 4, the 49 remaining females fell into two sharply defined groups when dif-

ferentiated by their mode of parthenogenetic reproduction. One, consisting of 34 females from the remaining 14 regions, produced a total of 739 progeny which, with the exception of one individual, spun small-sized cocoons and eventually developed into adult males; this group therefore consists of facultative females. The exceptional larva spun a large cocoon in the moss from which a female subsequently emerged; as will be shown later (p. 282) it probably resulted from accidental admixture and will not be considered here.

The other group consisted of the remaining 15 females which, however, were restricted to 6 of the regions tested; they produced 121 females and 2 males and thus are obligatory parthenogenetic forms.

TABLE 4.—*D. polytomum* REARING SUMMARY

Locality	Families raised	Family numbers	Males spun in		Females spun in		Total
			Foliage	Moss	Foliage	Moss	
1st generation							
Spindelmuhle	11	1–11	104	238	0	1*	342
Klenec	2	1–2	40	22	0	0	62
Dobris	1	1	4	0	0	0	4
Hvozdany	1	1	7	0	0	0	8
Myto	8	1–8	60	88	0	0	148
Kout	2	1–2	27	14	0	0	41
Klatovy	1	1	19	13	0	0	32
Stepanov	1	1	6	0	0	0	6
Kunzak	1	1	2	11	0	0	13
Plese	1	1	2	3	0	0	5
Albrechtice	1	1	7	0	0	0	7
Jenikov, 600m.	1	1	7	1	0	0	8
Jenikov, 400–500m.	2	2–3	26	25	0	0	51
Brezno	1	1	12	0	0	0	12
Total	34		323	415	0	1*	738
Ruzomberok.	1	1	0	1†	0	0	1
Klenec	4	3–6	0	0	0	24	24
Myto	1	9	0	0	0	14	14
Kout	2	3–4	0	0	0	13	13
Klatovy	3	2–4	0	0	0	32	32
Kunzak	1	2	0	0	0	13	13
Jenikov, 400–500	4	4–7	0	2	0	25	27
Total	15		0	2	0	121	123
Canada	3	1–3	0	0	2	16	18
2nd generation							
Klenec	5 ex 3	7–11	0	0	0	14	14
Myto	1 ex 9	10	0	0	0	5	5
Kunzak	1 ex 2	3	0	1	0	2	3
Total	7		0	1	0	21	22
Grand total	60		323	419	2	158	902

* Female excluded from totals (see above).
† Parthenogenetic status uncertain (see text p. 264).

Three of the first 25 families raised were bred from females selected especially for a particular body colour; they were facultative. The other 22 were from females set up in the rearing cylinders in the order in which they emerged from samples taken at random from the stored material; 19 proved to be facultative and 3 obligatory. In contrast, when only the largest cocoons were selected, as with the last 24 females bred, they were found to consist of 12 facultative and 12 obligatory individuals. Since the material from 8 regions was exhausted before the size selection tests were initiated, the last 24 rearings were necessarily restricted to females from the remaining 6 regions.

In order to determine whether there are morphological differences between the adult females of the 2 parthenogenetic types, the parents and part of the progeny were preserved for comparative examination; the results are given in Section VII.

That the obligatory females were generally larger than the facultative females is clear from the above data concerning their respective frequencies among selected as opposed to unselected females. When the hypothesis that selection for greater size leads to an increase in the proportion of obligatory forms is tested by means of a fourfold table, it is found that $X^2 = 6.91$, which with one degree of freedom gives a value[3] for P of slightly less than 0.01. The hypothesis is thus clearly in accord with observation.

In the larval stages two distinct forms occurred among the male progeny of certain females from Spindelmuhle and the Jenikov 400–500 metre collection. The one was normal in appearance in having a basic green colouration upon which a white pattern was superimposed. The other, though identical in the early instars, had in the fourth and fifth a salmon-red ventro-lateral stripe running the whole length of the body, a salmon-red underside to the abdomen, and only faint markings over the bases of the prolegs; these are called "red" larvae. The colouring on both the dorsal and lateral sides disappears at the fifth moult, that on the ventral side alone remaining throughout the prepupal stage into the pupal stage.

Upon dissecting the remaining material it was found that among the Spindelmuhle cocoons, in addition to *D. abieticolor*, 2 types of prepupae occurred. One was normal in appearance; the other was of a uniform dull green colour except for a distinct red colouring on the ventral surface of the abdomen. Three of the latter were set up separately in cylinders and from them 77 larvae were bred which had in every instance a red pigmentation distributed in the fourth and fifth instars as described above; their mothers were therefore homozygous reds. In addition 8 Spindelmuhle females which developed from green prepupae have been tested. From 3 a total of 91 green larvae was obtained demonstrating that the mothers were homozygous greens. The remaining five, however, were found to segregate for colour into greens and reds in the proportion of 100 to 74; the mothers in this group were thus heterozygous greens.

The heterozygous green female found in the Jenikov 400–500 metre collection produced 8 green and 13 red larvae. Combining the progeny

[3] The Ruzomberok female was from unselected material; its inclusion with the obligatory forms gives a X^2 of 5.5 and P equal to 0.02 –the conclusion therefore remains unaltered.

from the two collections it is seen that the segregating greens produced a total of 108 green and 87 red larvae. No red larvae were found among the progeny of obligatory parthenogenetic females.

In the early instars the obligatory larvae are quite indistinguishable both from their own rare brothers and from the green male progeny of facultative females. The two sexes appear then to be about the same size and as nearly as can be judged seem to exhibit the same head and body colour—a uniform brownish-green body with the head-capsule and eyes entirely black. Even in the fourth and fifth instars, when a definite body pattern is attained, they nevertheless resemble each other sufficiently closely to mask any slight differences that may possibly be present. Further, the obligatory females are indistinguishable from Canadian females and the facultative males are identical with the rare obligatory males. The only recognizable difference between the two types of European progeny in the larval stage is one of gross size, but this does not become pronounced until the fourth instar. In the fifth it is even more marked for the females have a mean length of 22.4 mm. (Table V) against approximately 15 mm. for the males. The differences in the mean length of obligatory female larvae from different regions are probably not significant as the error in the measurement of live larvae may possibly have been greater than the observed differences. However, it is almost certain that the larvae are as long as the Canadian females, if not longer.

TABLE 5.—MEAN FIFTH INSTAR LENGTH IN MM. FOR THE OBLIGATORY FORMS FROM CANADA AND CZECHOSLOVAKIA REARED UNDER IDENTICAL CONDITIONS

Family	Length of one larva	Mean length per region	Mean length per country
		mm.	mm.
Canada, 1	22.5		
2	23.0	22.2	22.2
3	21.0		
Klenec, 3	22.5		
4	22.0		
5	22.5		
6	23.0		
7 ex 3	22.5	22.4	
8 ex 3	22.0		
9 ex 3	22.0		
10 ex 3	22.5		
11 ex 3	22.5		
Myto, 9	22.5		
10 ex 9	23.0	22.8	
			22.4
Jenikov, 4	22.5		
5	22.0		
6	23.0	22.4	
7	22.0		
Kout, 3	22.5		
4	23.0	22.8	
Klatovy, 2	22.0		
3	22.5	22.0	
4	21.5		

Although the results of larval measurements fail to supply conclusive evidence for the occurrence of definite differences between the obligatory form from different localities, the evidence from the more accurate cocoon weighings is more conclusive. When the means for the five European regions are compared with that for the Canadian form (Table VI), the cocoons from Myto and Kout appear to be significantly heavier, those from Jenikov possibly heavier, while the Klenec and Klatovy means are approximately the same as the Canadian mean.

TABLE 6.—MEAN COCOON WEIGHT IN GRAMS FOR OBLIGATORY FAMILIES FROM CANADA (C) AND CZECHOSLOVAKIA (E) REARED UNDER IDENTICAL CONDITIONS

Family	No. cocoons per family	Mean weight per family	Mean weight per region	Difference between means	Significance
		gm.			
Canada, 1	3	0.066			
2	10	0.060	0.061	$M_E - M_C = 0.004$	±0.001
3	3	0.061			
Klenec, 3	4	0.058			
4	8	0.059			
5	1	0.067			
6	3	0.059			
7 ex 3	3	0.067	0.061	$M_K - M_C = 0.000$	±0.001
8 ex 3	2	0.064			
9 ex 3	4	0.061			
10 ex 3	3	0.062			
11 ex 3	2	0.064			
Myto, 9	9	0.079			
10 ex 9	4	0.083	0.080	$M_M - M_C = 0.019$	±0.001
Jenikov, 4	3	0.064			
5	5	0.065			
6	7	0.075	0.065	$M_J - M_C = 0.004$	±0.002
7	7	0.065			
Kout, 3	9	0.068			
4	1	0.070	0.068	$M_K - M_C = 0.007$	±0.002
Klatovy, 2	10	0.060			
3	10	0.061	0.060	$M_K - M_C = 0.002$	±0.002
4	6	0 057			

It has already been mentioned that in Canada the larvae almost always spin their cocoons in the moss and litter below the trees, while in Europe the majority of cocoons are spun in the lower foliage or surrounding herbaceous growth. In the laboratory rearings an attempt has therefore been made to simulate the natural conditions by offering the larvae the alternative of spinning either in the foliage or in damp moss placed on the bottom of the rearing cylinders. As is shown in Table 7, although 142 females and 3 males from obligatory females were in every instance spun deep in the moss[4], the spinning positions of the male progeny of facultative females were far from uniform. In the different facultative

[4] The single male progeny from Ruzomberok spun its cocoon in the foliage.

TABLE 7.—COCOON SPINNING POSITION AND PERCENTAGE LARVAL MORTALITY

Family		Cocoons in		Cocoons in foliage		Number of		Larval mortality	
		Fol.	Moss	Per fam.	Per local.	Larvae	Cocoons	Per fam.	Per local.
				%	%			%	%
Dobris	1	4	0	100.0	100.0	?	4	?	?
Hvozdany	1	7	0	100.0	100.0	?	7	?	?
Stepanov	1	6	0	100.0	100.0	?	6	?	?
Albrechtice	1	7	0	100.0	100.0	?	7	?	?
Brezno	1	12	0	100.0	100.0	?	12	?	?
Jenikov	1	7	1	87.5	87.5	8	8	0.0	0.0
Kout	1	13	4	76.5		17	17	0.0	
	2	14	10	58.3	65.8	53	24	54.7	41.4
Klenec	1	20	8	71.4		40	28	30.0	
	2	20	14	58.8	64.5	42	34	19.0	24.4
Klatovy	1	19	13	59.4	59.4	38	32	15.8	15.8
Jenikov	2	17	13	56.7		49	30	38.8	
	3	9	12	42.8	51.0	27	21	22.2	32.9
Myto	3	16	4	80.0		54	20	63.0	
	1	17	13	56.7		33	30	9.1	
	8	10	8	55.6		26	18	30.8	
	6	1	1	50.0		2	2	0.0	
	5	8	21	27.6	40.5	37	29	21.6	31.2
	4	4	16	20.0		29	20	31.0	
	7	2	8	20.0		14	10	28.6	
	2	2	17	10.5		20	19	5.0	
Plese	1	2	3	40.0	40.0	9	5	44.4	44.4
Spindelmuhle	1	18	12	60.0		36	30	16.7	
	4	12	15	44.4		30	27	10.0	
	3	7	9	43.8		20	16	20.0	
	9	13	17	43.3		36	30	16.7	
	6	7	13	35.0		22	20	9.1	
	8	10	25	28.6	30.4	49	35	28.6	16.6
	7	11	28	28.2		43	39	9.3	
	2	11	34	24.4		59	45	23.7	
	11	11	34	24.4		53	45	15.1	
	10	4	21	16.0		25	25	0.0	
	5	0	30	0.0		37	30	18.9	
Kunzak	1	2	11	15.4	15.4	14	13	7.1	7.1
		323	415	Mean = 51.04 ±4.91		922	702*	Mean = 20.32 ±2.88	
Kout	3	0	12	0.0		14	12	14.3	
	4	0	1	0.0	0.0	2	1	50.0	18.8
Klenec	3	0	9	0.0		15	9	40.0	
	4	0	8	0.0		17	8	52.9	
	5	0	2	0.0		4	2	50.0	
	6	0	4	0.0		12	4	66.6	
	7 ex 3	0	3	0.0	0.0	4	3	25.0	45.7
	8 ex 3	0	3	0.0		3	3	0.0	
	9 ex 3	0	4	0.0		6	4	33.3	
	10 ex 3	0	3	0.0		7	3	57.1	
	11 ex 3	0	2	0.0		2	2	0.0	
Klatovy	2	0	11	0.0		12	11	8.3	
	3	0	13	0.0	0.0	29	13	55.2	38.5
	4	0	8	0.0		11	8	27.3	
Jenikov	4	0	5	0.0		15	5	66.6	
	5	0	6	0.0		7	6	14.3	
	6	0	9	0.0	0.0	21	9	57.1	57.8
	7	0	7	0.0		21	7	66.6	
Myto	9	0	14	0.0		15	14	6.7	6.7
	10 ex 9	0	5	0.0	0.0	?	5	?	
Kunzak	2	0	13	0.0		?	13	?	
	3 ex 2	0	3	0.0	0.0	13	3	76.9	76.9
		0	145	Mean = 0.0		230	127*	Mean = 38.41 ±5.40	

* Excluding families with undetermined number of larvae.

TABLE 7.—COCOON SPINNING POSITION AND PERCENTAGE LARVAL MORTALITY—*Concluded*

Family		Cocoons in		Cocoons in foliage		Number of		Larval mortality	
		Fol.	Moss	Per fam.	Per local.	Larvae	Cocoons	Per fam.	Per local.
				%	%			%	%
Canada	1	0	3	0.0		5	3	40.0	
	2	2	10	16.6	11·1	27	12	55.5	50.0
	3	0	3	0.0		4	3	25.0	
		2	16	Mean = 11.11		36	18	Mean = 50.0	

families the percentage of cocoons spun in the foliage varied from 100 to 0, the mean for all being 51.04 ± 4.91%. This mean, however, is weighed down considerably by the Spindelmuhle families which have a mean of only 30.4%. Moreover, it was noted that the facultative cocoons were frequently spun on the moss or only just below the surface, so that unlike the obligatory females the tendency to burrow is evidently not very strong.

The small size of the families produced by the obligatory females when compared with those produced by facultative females is very striking. The 34 facultative females gave an average of 21.71 ± 1.98 cocoons, while 22 obligatory females averaged 6.59 ± 0.83 cocoons. Since the difference is very significant (15.12 ± 2.15) it remained to determine whether the difference is the result of a difference in the number of eggs laid by the 2 forms or whether it is due to differential egg mortality, larval mortality or both. It is naturally difficult to determine by direct observation the number of eggs laid, as oviposition continues for 2 or 3 days. At the completion of oviposition an attempt had been made to count the number of slits in the spruce needles, but owing to visual difficulties this proved uncertain. An estimate was then attempted by counting the number of nidal pellets, but it was found that this also was inaccurate since the pellets frequently adhered to the foliage and consequently remained obscured, while on occasions the egg was not inserted in the receptacle thus prepared. An estimate was finally arrived at by counting the number of larvae which hatched (this was not done for the families denoted by the question mark). Data concerning the larval frequencies are given in Table 7. Comparing the 2 forms it was found that the facultative females produced an average of 31.79 ± 2.77 first instars against 11.50 ± 1.58 for the obligatory females. With a difference between the means of 20.29 and a standard error of only 3.19, the difference is clearly significant. Further, there is a greater post-embrionic mortality among the obligatory progeny than is the case with facultative progeny. When the mean mortality is calculated from the percentages for the different families it is found to be 20.32 ± 2.88 for the facultative form and 38.41 ± 5.40 for the obligatory. The difference between the means being 18.09 and the standard error 6.12, it is probable that the obligatory larvae have suffered a greater mortality.

Again there was a significant difference between the 2 forms in the mean length of the total developmental period from oviposition to the spinning of the cocoon. Under the laboratory conditions mentioned the time taken was 27.62 ± 0.48 days for the obligatory families as opposed

to 20.55 ± 0.29 days for facultative families[5]. The difference, 7.07 days, has a standard error of 0.56 and is therefore highly significant. The greater mortality in the case of the obligatory form may thus be due in part to its longer period of development.

Data on diapause are difficult to analyse, partly due to the variable nature of the tendency (Balch (1)), but mainly perhaps owing to enforced changes in such environmental factors as temperature and humidity (brought about in transporting the reared material from one laboratory to another), the effect of which can not be readily estimated. The temperature changes are probably mainly responsible for the high post-larval mortality, for if development starts just prior to returning the material to cold storage the sudden chilling appears to be lethal. However, since the facultative and obligatory forms have suffered equally in this respect, 36.2 and 35.1% respectively, mortality can be disregarded in a consideration of diapause.

Of the material which survived, 3.0% of the facultative larvae and 14.1% of the obligatory larvae have failed to resume development after at least 3 periods of exposure to warmth during the year after the cocoons were spun. The 3.0% of facultative larvae, however, were all from the Spindelmuhle families (Table 12), whereas larvae in diapause occur in all the obligatory families except those from Klatovy, and are most frequent in the Myto families. Whether the diapause tendency is strengthened by exposure to cold is not clearly proved, but it was found that when larvae were kept in the warm rearing room continuously they developed directly into adults. Unfortunately the numbers tested (8♂♂ and 10♀♀) were not large enough to give a conclusive answer to this question.

D. polytomum—Canada

The 3 families raised for comparative purposes came from material collected in the immediate vicinity of Fredericton, N.B. The total number of larvae produced by the 3 mothers was 36, of which only 18 reached the cocoon stage to give a mean mortality of 50%. Sixteen of the cocoons were spun in the moss and 2, or 11.11%, were spun in the foliage (Table 7). Apart from this there is close agreement with the European obligatory families where the mean number of cocoons is 6.6 and the mean mortality 38.41%.

As mentioned earlier, the larvae cannot be distinguished from those of the European obligatory form either in body pattern or size, but the mean weight of the spun sixth instar larvae may possibly be significantly different from those of some European localities (Table 6). However, they show approximately the same diapause tendency (Table 12).

D. abieticolor, D.T.

Material of this species, which also feeds on *Picea*, occurred freely in the collections of *D. polytomum* from Jenikov, Kout, Myto and Spindelmuhle. The summarized rearing records in Table 8 show the species to be facultatively parthenogenetic.

[5] The developmental period for the single male progeny of the aberrant Ruzomberok female was 26 days

In the first and second instars the larvae can readily be distinguished from those of *D. polytomum* by means of the head-capsule which, apart from the dark eyes, is uniformly pale gold. The general body colour, however, is very similar in the early instars, and it is not until the fourth that it takes on its characteristic pattern. It is then khaki brown with chocolate spots, and thus differs markedly from *D. polytomum*. At the end of the fifth instar the larva moults and assumes a light brown colour on the dorsal and lateral surfaces, but the underside of the abdomen is suffused with pink. The cocoons are invariably spun in the moss and differ in this way from the progeny of facultatively parthenogenetic *D. polytomum*. In appearance the cocoons of the 2 species are indistinguishable.

The 5 families reared produced an average of 32.4 first instars of which 17.8 reached the cocoon stage; the mortality was therefore 45.06%. In comparison with the facultative families of *D. polytomum* we see that although approximately equal numbers of larvae were initially produced by the 2 species, the mortality in the base of *D. abieticolor* was markedly higher.

TABLE 8.—*D. abieticolor* AND *D. nemorum* REARING SUMMARY

Locality	Family number	First instar larvae produced	Number cocoons in		Larval mortality	
			Foliage	Moss	Per local.	Per species
					%	%
D. abieticolor:						
Jenikov	1	34	0	28		
	2	32	0	11	40.91	
Myto	1	43	0	31	27.91	45.06
Kout	1	21	0	16	23.81	
Spindel-muhle	1	32	0	3	90.62	
		162	0	89		
D. nemorum:						
Sweden		3	0	2	33.33	33.33

The mortality after cocoon spinning is even higher than during active larval life, for 67.4% succumbed during the later stage. Of the remainder, 17.2% remained dormant, but 4 developed directly when kept at room temperature.

D. nemorum, F. von Gmel.

Only one female of this species was available; it occurred in a collection of *D. simile*, Htg. from Sweden. The cocoons are quite different from those of *D. polytomum* being extremely tough and slightly purple in colour. They are somewhat larger and more oval than those of *D. simile* which in turn differ similarly from those of *D. polytomum*. Again, the eggs, like those of *D. simile*, are laid in the needles of various species of *Pinus* but they differ in distribution by being laid not in contiguous groups but singly.

Only 4 eggs were laid and from these only 3 larvae hatched (Table 8). In the later instars the larvae differ considerably from the other species reared. In the fourth, for example, the body is almost uniformly dull green except for a yellowish tinge towards the head and anus. There are however small light yellow areas around the spiracles in the abdominal region. The sclerotized regions of the legs are jet black, the non-sclerotized parts grey-green. In the fifth instar the body is divided into 5 longitudinal regions of approximately the same width which consist of 3 dull green stripes separated by 2 yellow-green stripes. The continuity of the stripes is interrupted by irregular jet black rings extending from the head to the anus. Other details also aid in making it totally unlike the larvae of other species examined.

Two of the larvae reached the sixth instar but although they both descended to the bottom of the rearing cylinder only one spun a cocoon; it was placed in cold storage and developed into an adult male soon after it was removed. The other developed directly into an adult male. This species, therefore, is facultatively parthenogenetic and is probably a non-diapause form.

VI. CYTOLOGY OF THE PROGENY OF D. POLYTOMUM

Facultative Males

In order to establish the relationship existing between chromosome number and type of parthenogenesis, at least one male from each progeny has been examined. Details are given in Table 9 and illustrations of many constitute most of Figures 36 to 65. Without exception the chromosome number was found to be six, that is, the number already established for the facultative males from Hradec u Opava. In Figures 36 to 65 the chromosomes of the complement taken from the brain tissue have been arranged in accordance with their probable homology as assumed from their gross morphology. In general they constitute 6 fairly distinct types: 1 large and 1 small each with a more or less median constriction; 2 with subterminal constrictions; and 2 medium sized members with sub-median constrictions. Upon closer examination, however, certain small but nevertheless definite modifications are apparent in one or more members of the complement. Whether these differences are sufficient to indicate the existence of distinct races is problematical, but it is significant that attempted mating proved unsuccessful although *D. simile* mates readily and successfully in captivity. As is to be expected no difference is visible between the chromosomes of green and red males coming from the same mother (Figures 45 *vs.* 46, and 59 *vs.* 60).

In presenting the Hradec u Opava observations (Section IV) it was mentioned that polyploid nuclei were frequent among somatic cells. There the highest multiple complexes observed were an octoploid nucleus in the ovariole wall and a *ca* 16-ploid cell in the brain. In the present material, nuclei with a much higher number of chromosomes were often seen. In one particular preparation from the brain of a male, hypodermal nuclei occurred which were approximately 20 to 30-ploid but the chromosomes were so numerous as to render it impossible to obtain more than a rough estimate of their actual number.

FIGURE

36. Green ♂, Kuusama, Rukatunturi, Finland.....
37. Green ♂, Pallastunturi, Finland..
38. Green ♂, Vittangi, Sweden.....
39. Green ♂, Spindelmuhle, north Bohemia, 1.....
40. Green ♂, Spindelmuhle, north Bohemia, 2.....
41. Red ♂, Spindelmuhle, north Bohemia, 5.....
42. Red ♂, Spindelmuhle, north Bohemia, 7.....
43. Green ♂, Spindelmuhle, north Bohemia, 8.....
44. Green ♂, Spindelmuhle, north Bohemia, 9.....
45. Green ♂, Spindelmuhle, north Bohemia, 10.....
46. Red ♂, Spindelmuhle, north Bohemia, 10.....
47. Green ♂, Spindelmuhle, north Bohemia, 11.....
48. Green ♂, Klenec, north Bohemia, 1
49. Green ♂, Klenec, north Bohemia, 2
50. Green ♂, Myto, mid Bohemia, 4..
51. Green ♂, Myto, mid Bohemia, 7..

FIGURES 36 to 65. The neural chromosomes of various European facultative males arranged in the order of their probable homology.

FIGURE

52. Green ♂, Hvozdany, south Bohemia, 1.....
53. Green ♂, Kout, south Bohemia, 2.
54. Green ♂, Klatovy, west Bohemia, 1
55. Green ♂, Albrechtice, north Moravia.....
56. Green ♂, Hradec u Opava, north Moravia.....
57. Green ♂, Jenikov, west Moravia, 1
58. Green ♂, Jenikov, west Moravia, 2
59. Green ♂, Jenikov, west Moravia, 3
60. Red ♂, Jenikov, west Moravia, 3..
61. Green ♂, Bystra, north Slovakia .
62. Green ♂, Lubochna, north Slovakia
63. Green ♂, Cervena Skala, mid Slovakia.....
64. Red ♂, Bogdan, Ruthenia.....
65. Red ♂, Paltnis, Roumania.....

FIGURES 36 to 65—*Continued.* The neural chromosomes of various European facultative males arranged in the order of their probable homology.

TABLE 9.—SUMMARY OF CHROMOSOME COUNTS IN FACULTATIVE PROGENY

Family		Larval colour	Number examined	Chromosome number		Figure
				Somatic	Meiotic	
Spindelmuhle	1	Green	5	n=6	n=6	39
	2	Green	2	n=6		40
	3	Green	2	n=6		
	4	Red	2	n=6	n=6	
	5	Red	3	n=6	n=6	41
	6	Red	2	n=6	n=6	
	7	Green	2	n=6	n=6	
		Red	2	n=6	n=6	42
	8	Green	1	n=6		43
		Red	1	n=6	n=6	
	9	Green	3	n=6		44
		Red	1	n=6		
	10	Green	1	n=6		45
		Red	1	n=6		46
	11	Green	1	n=6		47
		Red	1	n=6		
Klenec	1	Green	4	n=6	n=6	48
	2	Green	2	n=6	n=6*	49 66A and 66B,
Dobris	1	Green	2	n=6		
Hvozdany	1	Green	2	n=6	n=6*	52
Myto	1	Green	2	n=6	n=6	
	2	Green	1	n=6		
	3	Green	4	n=6	n=6	
	4	Green	1	n=6		50
	5	Green	4	n=6	n=6	
	6	Green	1	n=6		
	7	Green	1	n=6		51
	8	Green	2	n=6		
Kout	1	Green	1	n=6		
	2	Green	4	n=6	n=6	53
Klatovy	1	Green	4	n=6	n=6	54
Stepanov	1	Green	1	n=6		
Kunzak	1	Green	1	n=6		
Plese	1	Green	2	n=6	n=6	
Albrechtice	1	Green	1	n=6		
Jenikov	1	Green	1	n=6		57
	2	Green	1	n=6		58
	3	Green	1	n=6		59
		Red	1	n=6		60
Brezno	1	Green	1	n=6		
		Green	61			
		Red	14			

* Plus a few diploid spermatocytes.

In no instance had this chromosome doubling been found to affect the germinal line nuclei until 2 of the reared males were examined. In these smear preparations the spermatocyte nuclei of perhaps a single loculus of the testis were found to possess the diploid number of chromosomes (Figure 66A *vs.* 66B). It was found that they followed precisely the same course as that of the normal haploid ones; they condense without pairing, congress rather loosely on the equatorial plate, and all 12 pass to the same pole at the first anaphase. At the second, they divide equationally and thus give rise to 2 daughter nuclei each with 12 chromosomes. This is conclusive evidence that the customary suppression of meiosis is a sexual

characteristic not determined directly by the haploid constitution of the male. The mechanism of meiosis must instead be genetically adapted to give regular failure of reduction.

Though diploid spermatocytes have previously been reported by Torvik-Greb (51) in diploid hymenopteran males originating as biparental offspring following inbreeding, this, to the best of the writer's knowledge, is the first report of spermatocytes diploid as the result of simple chromosome doubling. Though the difference between the two cases is not very great it is at least certain that in the present instance homozygosity must be absolute, and the behaviour reported is therefore of importance to an understanding of the origin of the hymenopteran type of spermatogenesis.

Obligatory Females

With the exception of the Kunzak families, at least 1 female from each of the obligatory families has been examined. The chromosome number determined from neural tissue, oogonia or oocytes was invariably 14 (Table 10). In Figures 67 to 73 are depicted the neural chromosomes of

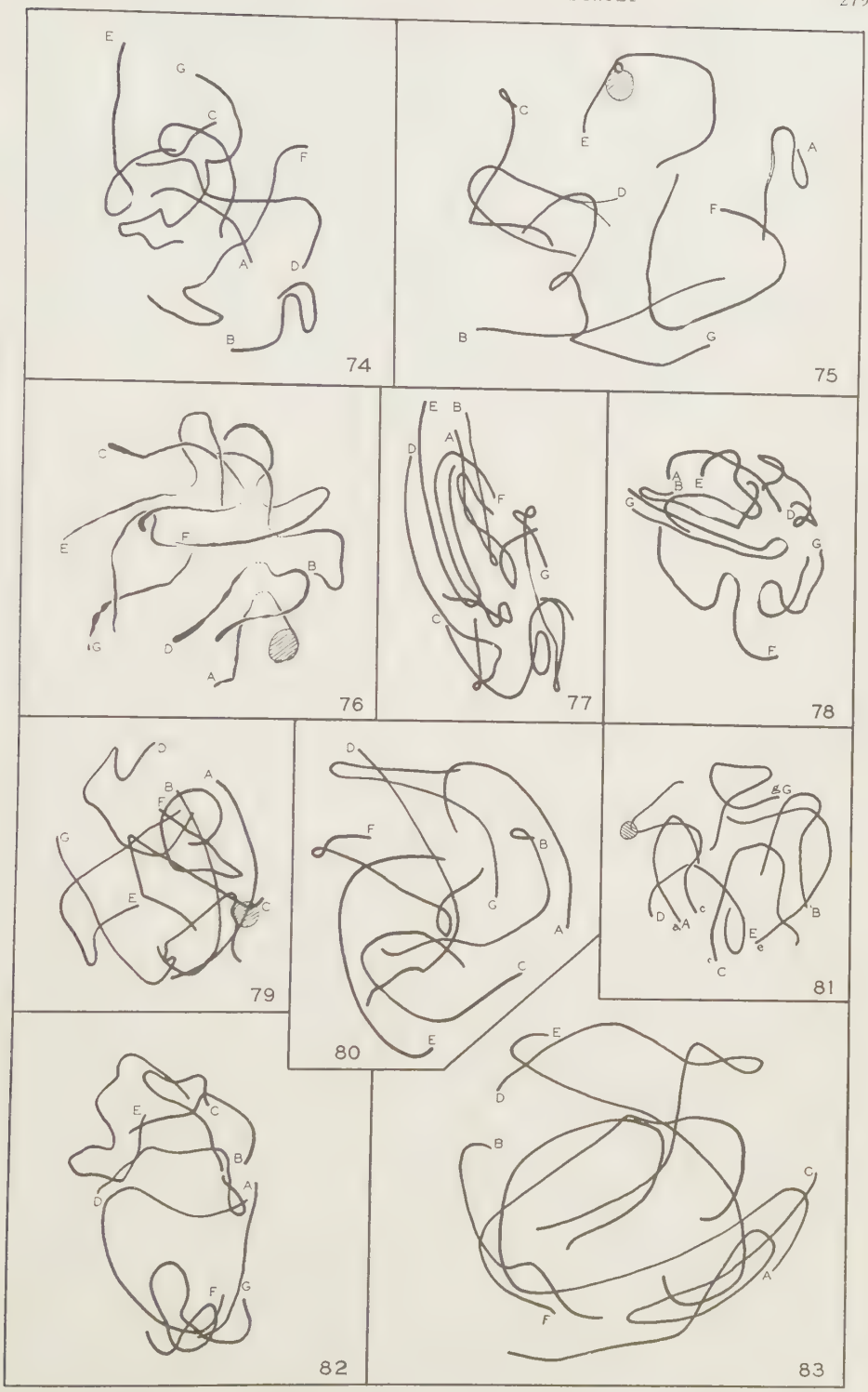
FIGURES 66A and B to 73. 66, Haploid and exceptional diploid spermatocytes of a facultative male; 67 to 73, somatic chromosomes of various obligatory progeny from Myto (neural), Jenikov (neural), Klenec (neural), Kout (neural prometaphase), Klatovy (neural), Myto (oogonial), and Myto (ovariole wall).

obligatory females from families from Myto, Jenikov, Klenec, Kout and Klatovy with the addition of illustrations of the somatic complements from an oogonium and the ovariole wall of a Myto female. Unfortunately, the chromosomes are too small and too numerous for indisputable identifica-

TABLE 10.—SUMMARY OF CHROMOSOME COUNTS IN OBLIGATORY PROGENY

Family	Number examined	Chromosome number		Figure
		Somatic	Meiotic	
Klenec, 3	1	2n = 14	—	
4	1	2n = 14	2n = 14	
5	1	2n = 14	2n = 14	69 and 76
6	1	2n = 14	—	
7 ex 3	1	2n = 14	2n = 14	
8 ex 3	1	—	2n = 14	
9 ex 3	1	2n = 14	2n = 14	
10 ex 3	1	2n = 14	—	
11 ex 3	1	—	2n = 14	
Myto 9	2	2n = 14	2n = 14	
10 ex 9	1	2n = 14	2n = 14	67, 72–74
Kout 3	2	2n = 14	2n = 14	
4	1	2n = 14	2n = 14	70 and 77
Klatovy 2	3	2n = 14	2n = 14	
3	1	2n = 14	2n = 14	71 and 78
4	2	2n = 14	2n = 14	
Jenikov 4	1	2n = 14	2n = 14	
5	1	—	2n = 14	75
6	1	2n = 14	2n = 14	68
7	1	—	2n = 14	
	25			
Canada, 2	2	2n = 14	2n = 14	
3	1	—	2n = 14	
	3			

tion, but it is evident that no striking variations are present. In Figures 74 to 78 the pachytene chromosomes of females from the same European sources are illustrated and compared with those of other species (Figures 79 to 81) and with those of the Canadian (Figure 82) and Hradec (Figure 83) *Diprion polytomum* females. Since the drawings have been taken from nuclei flattened with considerable pressure the chromosomes have not been optically foreshortened and thus direct measurement has supplied reliable data on the pachytene lengths of the respective members of the complement. These data are given in Table 11 where the relative lengths of the chromosomes are expressed as a percentage of the total length of the whole complement. It is clear that though in general the agreement is fairly close, there are in some instances relatively large discrepancies which may be due to actual length differences or to differential pachytene contraction of the whole or parts of the chromosomes. The agreement between the third Jenikov nucleus and the first Canadian nucleus is strikingly close but none agrees well with those of the other 3 species. The pachytene chromosomes of the latter 3 species agree reasonably well with the relative lengths of their respective male complements, so that it is probable that the differences observed between the various obligatory females are at least partly real linear differences. If this is so, then the differences may be taken to indicate the existence of genetically distinct lines or races of the obligatory *D. polytomum*. Such chromosome changes will naturally be perpetuated as a result of the obligatory mode of parthenogenetic reproduction.



FIGURES 74 to 83. 74 to 78, the pachytene chromosomes of obligatory progeny from Myto, Jenikov, Klenec, Kout and Klatovy respectively. 79 to 83, ditto of *D. abieticolor*, *D. nemorum*, *N. sertifer* and *D. polytomum* obligatory and facultative from Canada and Czechoslovakia respectively.

TABLE 11.—THE RELATIVE LENGTHS OF THE PACHYTENE CHROMOSOMES EXPRESSED AS A PERCENTAGE OF THE TOTAL LENGTH OF THE CHROMOSOMES

Species		Derivation	A	B	C	D	E	F	G
D. abieticolor	(F)	Ruzomberok	11.2	11.6	13.2	13.8	14.4	17.2	18.7
D. sertifer	(F)	Aryd, Sweden	10.9	13.7	14.2	14.6	15.3	15.4	15.9
D. nemorum	(F)	Finland	13.0	13.0	13.5	14.3	14.3	15.3	16.6
D. polytomum	(O)	Myto	7.3	12.1	13.4	14.1	15.6	17.9	19.5
	(O)	Klatovy	8.9	11.7	13.7	14.0	15.7	15.8	20.2
	(O)	Klenec	10.0	12.7	13.3	14.1	15.4	17.0	17.5
	(O)	Kout	11.0	12.7	14.1	14.9	15.3	15.6	16.4
	(O)	Jenikov	10.4	11.6	11.8	13.5	14.8	17.9	19.9
	(O)	Jenikov	11.4	13.0	13.4	14.6	15.8	15.8	16.1
	(O)	Jenikov	9.7	11.3	12.5	12.6	14.0	19.2	20.7
	(O)	Canada	9.6	11.3	12.4	12.6	13.8	19.0	21.2
	(O)	Canada	11.3	12.8	13.3	14.4	15.6	15.6	16.9
			A	B	C	D	E	F	
	(F)	Moravia	8.1	14.2	15.0	18.2	20.8	23.7	

VII. MORPHOLOGY OF PARENTS AND PROGENY

In order to determine whether the females with different types of parthenogenetic reproduction show correlated differences in their gross morphology, the parents have been retained either as pinned specimens or preserved in alcohol. These were then supplied with samples of their progeny to W. A. Reeks of the Dominion Entomological Laboratory, Fredericton, for a detailed morphological study. Each parent and each progeny received a number, but no information concerning them was supplied, so that not even the relationships were known. Mr. Reeks' detailed findings will be published later, but in view of their immediate interest he has permitted the inclusion of certain of the preliminary data here.

From an investigation of miscellaneous collections made in Czechoslovakia during 1936, Reeks (36) reached the conclusion that 2 or more morphological strains of *D. polytomum* might exist in Europe, of which 1 appeared to be similar to the Canadian form. Certain genital characters were mainly responsible for this theory but, although the males and females could each be separated into 2 distinct groups, there was no means, other than relative frequencies, of correlating the male with the female of each type. However, when related males and females became available from these rearing experiments in England, their comparison readily overcame this limitation.

Of a total of 59 *D. polytomum* parents preserved, 47 were suitable for examination; 6 were both first generation progeny and second generation parents; 2 of the parents were Canadian, 19 were European obligatory forms, and 26 were European facultative forms (Table 12). The Canadian parents were found to possess on each of the second pair of valvulae a process which was spiral in form (Type B genitalia), but they differed in the number of

TABLE 12.—SUMMARY OF MORPHOLOGY OF PARENTS AND PROGENY

Family		Family genitalia	Colour of progeny	Sex and no. of progeny examined with genitalia			Chromosome no. of progeny and number examined	Per cent failed to develop (diapause)
				Type A	Type ?	Type B		
Spindelmuhle	1	Type A	Green	8♂	1♂	1♀†	n=6 (5)	6.0
	2	A	Green	13♂	3♂	—	n=6 (2)	15.0
	3	A	Green	8♂	—	—	n=6 (2)	0.0
	4	A	Red	15♂	—	—	n=6 (2)	0.0
	5	A	Red	10♂	2♂	—	n=6 (3)	11.1
	6	A	Red	7♂	—	—	n=6 (2)	0.0
	7	A	Green	5♂	—	—	n=6 (2)	0.0
			Red	11♂	—	—	n=6 (2)	0.0
	8	A	Green	3♂	—	—	n=6 (1)	0.0
			Red	10♂	—	—	n=6 (1)	0.0
	9	A	Green	5♂	4♂	—	n=6 (3)	33.3
			Red	3♂	—	—	n=6 (1)	0.0
	10	A	Green	4♂	1♂	—	n=6 (1)	9.1
			Red	1♂	2♂	—	n=6 (1)	50.0
	11	A	Green	20♂	1♂	—	n=6 (1)	3.4
			Red	2♂	—	—	n=6 (1)	0.0
Klenec	1	Type ?*	Green	2♂	—	—	n=6 (4)	0.0
	2	A	Green	9♂	—	—	n=6 (2)	0.0
	3	B	Green	—	—	8♀	2n=14 (1)	0.0
	4	B	Green	—	—	2♀	2n=14 (1)	0.0
	5	B	Green	—	1♀	—	2n=14 (1)	50.0
	6	?	Green	—	—	1♀	2n=14 (1)	0.0
	7 ex 3	B	Green	—	—	—	2n=14 (1)	0.0
	8 ex 3	B	Green	—	—	—	2n=14 (1)	0.0
	9 ex 3	B	Green	—	1♀	—	2n=14 (1)	50.0
	10 ex 3	B	Green	—	—	—	2n=14 (1)	0.0
	11 ex 3	B	Green	—	1♀	—	2n=14 (1)	50.0
Myto	1	Type A	Green	16♂	—	—	n=6 (2)	0.0
	2	?	Green	5♂	—	—	n=6 (1)	0.0
	3	A	Green	4♂	—	—	n=6 (4)	0.0
	4	A	Green	8♂	—	—	n=6 (1)	0.0
	5	A	Green	14♂	—	—	n=6 (4)	0.0
	6	A	Green	—	—	—	n=6 (1)	0.0
	7	A	Green	6♂	—	—	n=6 (1)	0.0
	8	?	Green	6♂	—	—	n=6 (2)	0.0
	9	B	Green	—	1♂	2♀	2n=14 (2)	20.0
	10 ex 9	B	Green	—	3♀	—	2n=14 (1)	75.0
Kout	1	Type A	Green	3♂	—	—	n=6 (1)	0.0
	2	B?	Green	—	—	—	n=6 (4)	0.0
	3	B	Green	—	1♀	5♀	2n=14 (2)	12.5
	4	B	Green	—	—	—	2n=14 (1)	0.0
Klatovy	1	Type A	Green	11♂	—	—	n=6 (4)	0.0
	2	B	Green	—	—	6♀	2n=14 (3)	0.0
	3	B	Green	—	—	3♀	2n=14 (1)	0.0
	4	B	Green	—	—	3♀	2n=14 (2)	0.0
Kunzak	1	Type A	Green	7♂	—	—	n=6 (1)	0.0
	2	?	Green	—	—	—	2n=??	0.0
[illegible]	3 ex 2	?	Green	—	1♀	1♀ and 1♂	2n=??	33.3
Plese	1	Type A	Green	2♂	—	—	n=6 (6)	0.0
Jenikov	1	Type A	Green	4♂	—	—	n=6 (1)	0.0
	2	A	Green	9♂	—	—	n=6 (1)	0.0
	3	A	Green	2♂	—	—	n=6 (1)	0.0
			Red	8♂	—	—	n=6 (1)	0.0
	4	B	Green	—	—	1♀	2n=14 (1)	0.0
	5	B	Green	—	—	—	2n=14 (1)	0.0
	6	B	Green	—	1♀	—	2n=14 (1)	33.3
	7	B	Green	—	—	—	2n=14 (1)	0.0
Canada	1	Type B	Green	—	—	—	2n=??	0.0
	2	B	Green	—	1♀	1♀	2n=14 (2)	16.7
	3	B	Green	—	—	1♀	2n=14 (1)	0.0
Europe—29 facul. = 25A + 1B? + 3?				241♂♂	14♂♂	1♀†	n=6 (68)	3.0
Europe—22 oblig. = 19B + 0A + 3?				0♀	10♀♀	32♀♀ +1♂	2n=14 (25)	14.1
Canada— 3 oblig. = 2B + 0A + 1?				0♀	1♀	2♀♀	2n=14 (3)	12.5

* Type ? indicates specimen not examined or larvae in diapause.
† = Accidental admixture (see text).

saw-bands on the first pair; one had 12 on each valvula, the other had only 11 on each. Similarly the 19 European obligatory parents had spiralled processes but 7 had 12 bands while 12 had only 11 on each valvula. Of the facultative females 25 differed in having without exception 10 saw-bands and straight processes on the second pair of valvulae (Type A). The 26th, from which no count of the number of bands was possible, was classified as spiralled, but this exceptional classification is probably due to its poor state of preservation.

The 32 female progeny of European obligatory forms and the 2 from Canadian females so far examined invariably resemble their mothers in having spiralled valvular processes and either 11 or 12 saw-bands. A total of 241 male progeny of facultative females has so far been examined and without exception the aedeagus is spatulate in form (Type A). In contrast the aedeagus of all Canadian males and that of the only European obligatory male examined is consistently pedate in form (Type B).

The exceptional female which occurred along with 30 males as the progeny of a female from Spindelmuhle (family 1) was found to have 11 saw-bands and spiral valvular processes, that is, the Type B characteristics of the obligatory form. Since 8 males from this family have facultative type genitalia, the exceptional female, which must have been the daughter of a virgin female, is considered to have resulted from accidental admixture with an obligatory culture.

The exceptional Ruzomberok female was classified as Type B, a classification apparently inconsistent with her production of a single male progeny. When, however, the male was examined it also was found to belong to Type B. The morphological data thus indicate that we are dealing with an obligatory form which laid only 1 egg, the egg developing into an exceptional male. Cytological confirmation could not be obtained as both criteria cannot be used. But it will be seen in Table 13 that 1 female from this region has been examined cytologically and that it had 14 chromosomes, the obligatory number. The possibility nevertheless remains that this family may be a representative of a facultative form with genitalia indistinguishable from the obligatory form. It is for this reason that this family has previously been omitted from consideration.

VIII. DISTRIBUTION OF THE OBLIGATORY FORM IN EUROPE

With the association between type of parthenogenesis, morphology, and chromosome number satisfactorily established, a number of miscellaneous collections from various countries in Europe have been examined with a view to determining the distribution there of the obligatory form. The localities from which the collections were derived are listed in Table 13 together with the chromosome number of the males and females examined and the type of genitalia of other adults. From these their probable type of parthenogenesis is inferred. In certain cases the weight of the female cocoon and its contained larva is given; these were determined in order to investigate the relationship between chromosome number or morphology and gross size, since direct observation and size selection tests indicate that obligatory females are larger than facultative females. Table 13 there-

TABLE 13.—CHROMOSOME NUMBER AND GENITALIA TYPE FOR MISCELLANEOUS COLLECTIONS

Locality	No. examined	Sex	Cocoon weight	No. of chromosomes or genitalia type	Assumed type of partheno-genesis	Figure
			gm.			
WEIGHT OF FEMALE DETERMINED						
Kuusama, Juurikkavaara, Finland	1	♂		n=7	Oblig.	
	1	♀	.063	A?	Facult?	
Kuusama, Rukatunturi, Finland	2	♂		n=6	Facult.	36
	1	♀	.060	B?	Oblig?	
Salla, Finland	1	♂		n=6	Facult.	
	2	♀	.057	A?	Facult?	
	1	♀	.059	2n=12	Facult.	
	1	♀	.062	2n=12	Facult.	
Pallastunturi, Finland	2	♂		n=6	Facult.	37
	1	♀	.068	2n=12	Facult.	
Vittangi, Sweden	6	♂		n=6	Facult.	38
	2	♀	.055	B?	Oblig?	
	2	♀	.056	2n=12	Facult.	
	2	♀	.057	A?	Facult.	
	1	♀	.063	2n=12	Facult.	
Riesengebirge, 1400 m., Bohemia	2	♂		A	Facult.	
	2	♂		n=6	Facult.	
Padrt, mid Bohemia	2	♂		n=6	Facult.	
	1	♀	.055	A	Facult.	
	1	♀	.055	2n=14	Oblig.	
	1	♀	.058	2n=14	Oblig.	
Milicin, east Bohemia	1	♂		A	Facult.	
	1	♂		n=6	Facult.	
	1	♀	.049	2n=12	Facult.	
	2	♀	.050	A	Facult.	
Strmilov, south Bohemia	3	♂		A	Facult.	
	1	♀	.053	2n=14	Oblig.	
	2	♀	.055	B	Oblig.	
Predin, west Moravia	2	♂		A	Facult.	
	1	♀	.055	A	Facult.	
	1	♀	.055	2n=12	Facult.	
	1	♀	.058	A	Facult.	
	1	♀	.059	2n=12	Facult.	
Bystra, north Slovakia	1	♂		A	Facult.	
	1	♂		n=6	Facult.	61
	1	♀	.056	2n=12	Facult.	
	2	♀	.056	B	Oblig.	
	1	♀	.058	2n=12	Facult.	
Ruzomberok, north Slovakia	1	♀	.061	2n=14	Oblig.	
Lubochna, north Slovakia	1	♂		A	Facult.	
	1	♂		n=6	Facult.	62
	1	♀	.056	2n=14	Oblig.	
Biely Potok, north Slovakia	1	♂		n=6	Facult.	
	1	♀	.050	A	Facult.	
	1	♀	.070	2n=14	Oblig.	
Cervena Skala, mid Slovakia	2	♂		n=6	Facult.	63
	1	♀	.054	2n=12	Facult.	
	1	♀	.055	A	Facult.	
	1	♀	.055	2n=12	Facult.	
	1	♀	.059	2n=14	Oblig.	
Balog, south Slovakia	1	♂		n=6	Facult.	
Bogdan, Ruthenia	1	♂		n=6	Facult.	64
	1	♀	.062	2n=14	Oblig.	
Paltnis, Roumania	1	♂		n=6	Facult.	65
	1	♀	.049	2n=12	Facult.	

TABLE 13.—CHROMOSOME NUMBER AND GENITALIA TYPE FOR MISCELLANEOUS COLLECTIONS —*Concluded*

Locality	No. examined	Sex	Cocoon weight	No. of chromosomes or genitalia type	Assumed type of parthenogenesis	Figure
	24	♂♂		n=6	Facult.	
	10	♂♂		A	Facult.	
	1	♂		n=7	Oblig.	
	14	♀♀		2n=12	Facult.	
	12	♀♀		A	Facult.	
	8	♀♀		2n=14	Oblig.	
	7	♀♀		B	Oblig.	
LARGEST SELECTED						
Spindelmuhle, north Bohemia	1	♀		2n=12	Facult.	
Klenec, north Bohemia	1	♀		2n=12	Facult.	
	1	♀		2n=14	Oblig.	
Myto, mid Bohemia	2	♀		2n=12	Facult.	
	2	♀		2n=14	Oblig.	
Jenikov, west Moravia	2	♀		2n=12	Facult.	
	6	♀♀		2n=12	Facult.	
	3	♀♀		2n=14	Oblig.	
NO SELECTION						
Spindelmuhle, north Bohemia	9	♂		A	Facult.	
	1	♂		n=6	Facult.	
	2	♀		2n=12	Facult.	
Dobris, mid Bohemia	1	♀		2n=12	Facult	
Myto, mid Bohemia	2	♀		A	Facult.	
Klatovy, west Bohemia	1	♀		A	Facult.	
Plese, south Bohemia	1	♂		n=6	Facult.	
Albrechtice, north Moravia	1	♀		2n=14	Oblig.	
	1	♂		n=6	Facult.	55
Hradec u Opava, north Moravia	13	♂		n=6	Facult.	56
	11	♀		2n=12	Facult.	
Jenikov, west Moravia	1	♂		n=6	Facult.	
Brezno, north Slovakia	1	♀		2n=12	Facult.	
Ruzomberok, north Slovakia	1	♀		2n=12	Facult.	
	17	♂♂		n=6	Facult.	
	9	♂♂		A	Facult.	
	16	♀♀		2n=12	Facult.	
	3	♀♀		A	Facult.	
	1	♀		2n=14	Oblig.	

fore consists of three parts: the first, in which the weights of the female cocoons and contained larvae were determined; the second, in which the seemingly largest female cocoons were selected on the basis of gross size; and the third, in which no selection was practiced.

In the first group a total of 22 females of known weight was examined cytologically and found to include 2 chromosomal types; 8 had 14 chromosomes and therefore are assumed to be obligatory; the remaining 14 had 12 chromosomes and thus are presumably facultative. All the 14-chromosome females and 7 of the 12-chromosome females came from collections made in Czechoslovakia; the remaining 7 came from Finland, Sweden and Roumania.

Since males collected from the field should be predominantly of the facultative type it was not surprizing to find that of the 25 examined all but one had a complement of 6 chromosomes (as stated in Table 13, some of these are illustrated in Figures 36 to 65). The exceptional individual possessed 7 chromosomes, the number found in the Canadian male; it is therefore tentatively regarded as a rare male from a normally obligatory mother such as has been found occasionally in the field in Canada and in laboratory cultures of Czechoslovakian material. There is, however, the possibility of it being the male of a different species which resembles *D. polytomum* in the larval stages or even a male of a facultative form of *D. polytomum* possessing 7/14 chromosomes.

A further 10 males and 19 females in this group were examined by Reeks. All the males were identical in possessing Type A intromittent organs, but the females were of two types; 12 possessed facultative type genitalia and 7 the obligatory. Reeks noted, however, that though they fell into one type or the other, the Swedish and Finnish females were slightly atypical. Grouping the females identified by these two criteria it is found that the obligatory females have a mean cocoon weight of 0.058 ± 0.001 gm., the facultative females one of 0.056 ± 0.001 gm. The difference between the means is clearly not significant (0.002 ± 0.001 gm.).

In the second group only females were studied; 6 had 12 chromosomes and are therefore probably facultative, while 3 had 14 chromosomes and are probably obligatory.

In the third group no selection was practiced. The 17 males examined cytologically had the facultative complement of 6 chromosomes and the 9 examined morphologically had the facultative type of genitalia. Of the females, 3 had the facultative type of genitalia, 16 others had 12 chromosomes and only 1 was found with 14 chromosomes. Thus only one obligatory female occurred among the 20 selected at random.

IX. CYTOLOGY OF RELATED SPECIES

In order to determine whether 6 or 7 is the basic chromosome number for the genus, 4 closely related species of *Diprion* have been investigated (Table 14). They are all facultative forms. A further facultative species was examined which belonged to a nearby genus, namely *Neodiprion sertifer*. Finally, the chromosome number of *Pristiphora erichsoni*, the larch sawfly, was determined since it reproduces by obligatory parthenogenesis.

D. abieticolor, D. T.

Material of this species derived from 6 more or less distinct bioclimatic regions in Czechoslovakia was identical in showing 7 chromosomes in the male and 14 in the female. The neural chromosomes of the male are illustrated in Figure 84 and those of the female in Figure 85. The course of spermatogenesis (Figures 86 and 87) and the early stages of oogenegenesis are indistinguishable from those already established for the obligatory form of *D. polytomum*. The 7 pachytene bivalents of a female are shown in Figure 79 and compared with those of other species in Table 11.

D. pallidum, Kl.

This species of pine sawfly was obtained from Plese, Bohemia. Here again the male has 7 chromosomes, the female 14. Typical complements are illustrated in Figures 88 and 89.

D. nemorum, F. von Gmel.

This is the largest of the sawflies examined. The male has 7 chromosomes (Figure 90) and the female 14. The 7 pachytene bivalents are illustrated in Figure 80 and compared with those of other species in Table 11. Note that the pachytene lengths agree with the male somatic chromosomes in being more uniform than is the case with *D. polytomum*.

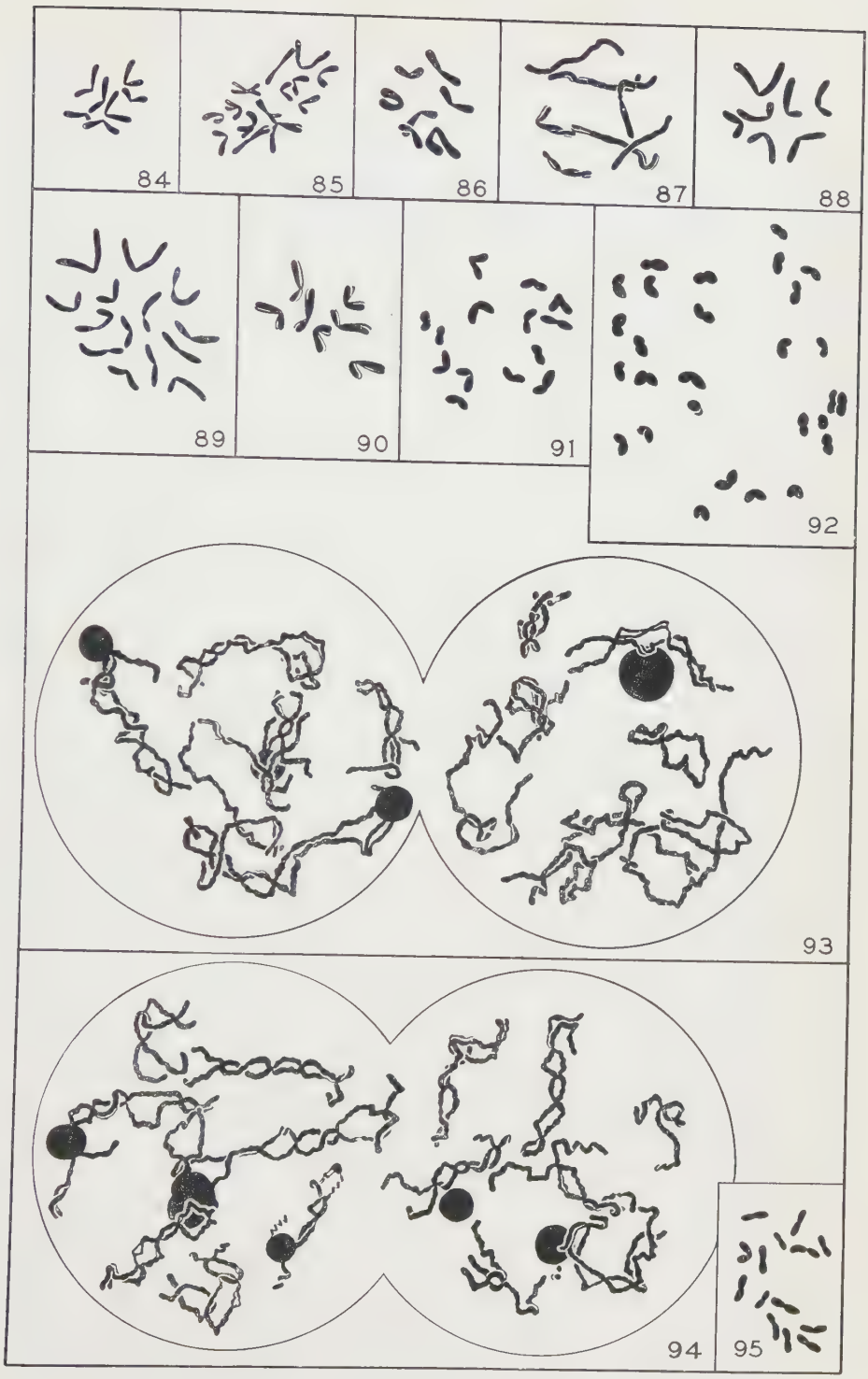
D. simile, Htg.

This species of pine sawfly is intermediate in size between *D. polytomum* and *D. nemorum*. In relation to the other species, however, it is diploid for the male sex and tetraploid for the female sex. The 14 spermatogonial chromosomes are shown in Figure 91, and the 28 oogonial chromosomes in Figure 92. Spermatogenesis follows the course typical for the Hymenoptera; 14 univalent chromosomes align themselves on the first metaphase plate and all pass to one pole at the first anaphase. Second anaphase consists of the separation of the split halves to form 2 telophase nuclei each with 14 chromosomes. That the failure to form bivalents is not to be considered as evidence that the 14 chromosomes consist of 2 non-homologous sets of 7 has earlier been shown in the case of aberrant diploid spermatocytes of *D. polytomum*.

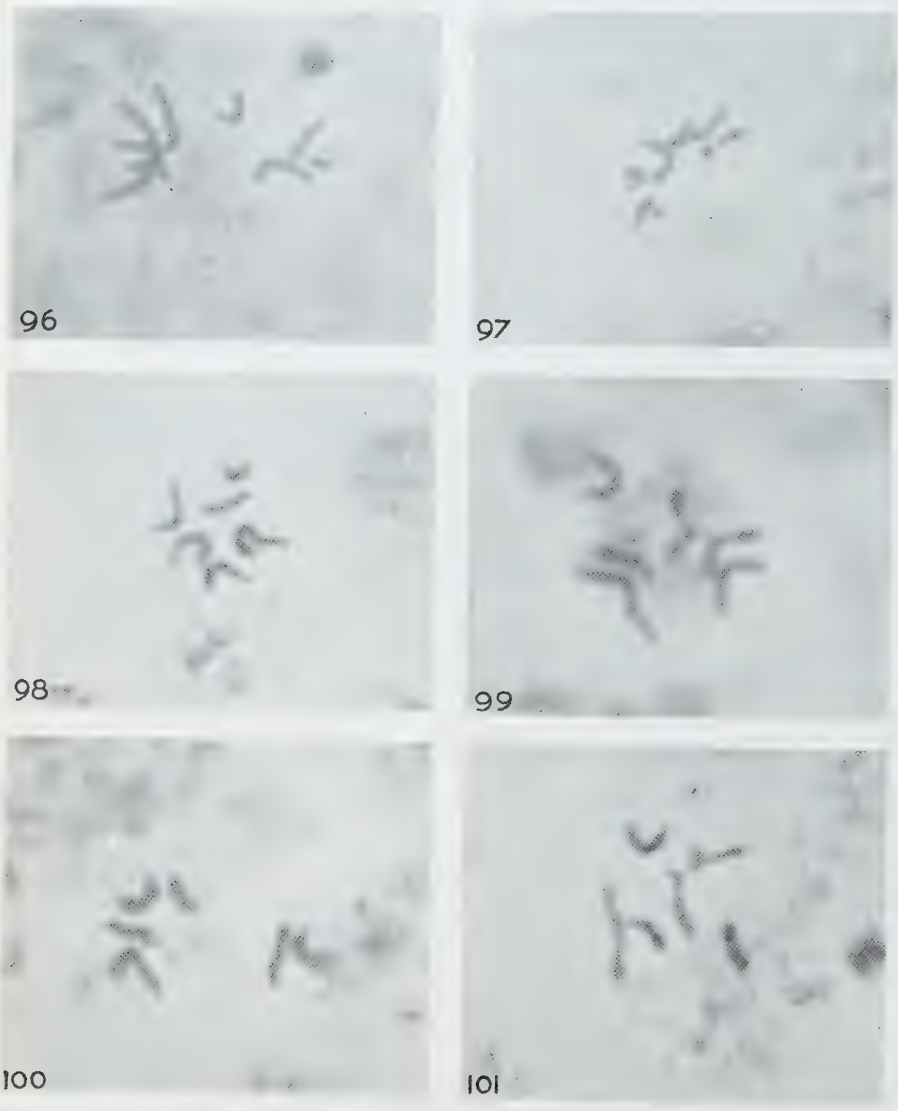
TABLE 14.—CHROMOSOME NUMBERS OF RELATED SPECIES

Species	Type of parthenogenesis	Derivation	Male		Female	
			Somatic	Meiotic	Somatic	Meiotic
D. abieticolor	Facul.	Biely Potok, Slovakia	7	—	—	—
		Jenikov, Moravia	—	7	—	14
		Klenec, Bohemia	7	7	14	14
		Myto, Bohemia	7	7	14	14
		Spindelmuhle, Bohemia	—	7	—	—
		Ruzomberok, Moravia	—	7	—	—
D. pallidum	Facul.	Plese, Bohemia	7	7	14	—
D. nemorum	Facul.	Finland	7	7	14	14
D. simile	Facul.	Canada	14	14	28	28
			—	14	—	28
N. sertifer	Facul.	Aryd, Sweden	7	7	—	14
P. erichsoni	Oblig.	Canada	—	—	14	—

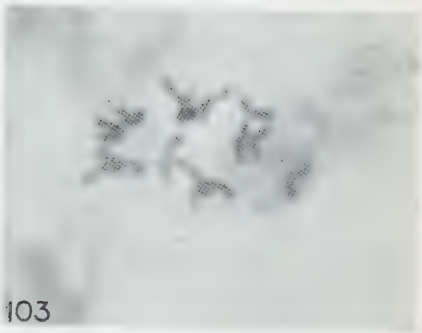
FIGURES 84 to 95. Somatic and meiotic chromosomes of related species: 84 and 85, the neural chromosomes of the male and female of *D. abieticolor;* 86 and 87, second metaphase and telophase in *D. abieticolor;* 88 and 89, the neural and ovariole wall chromosomes of the male and female of *D. pallidum;* 90, the neural chromosomes of the male *D. nemorum;* 91 to 94, *D. simile*—the 14 spermatogonial chromosomes of the male, the 28 oogonial chromosomes of the female, and 2 early diplotene nuclei in which the bivalents have been spaced for clarity; 95, the neural chromosomes of the obligatory species *P. erichsoni*.

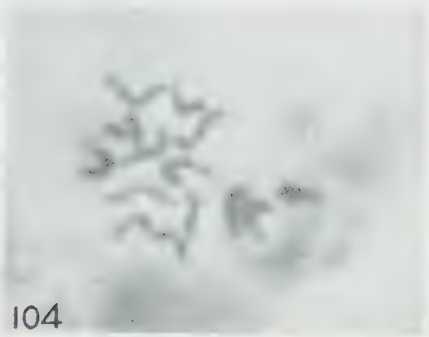


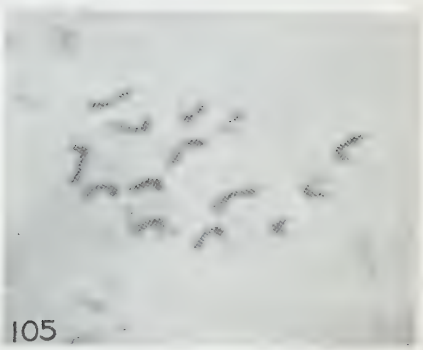
FIGURES 84 TO 95

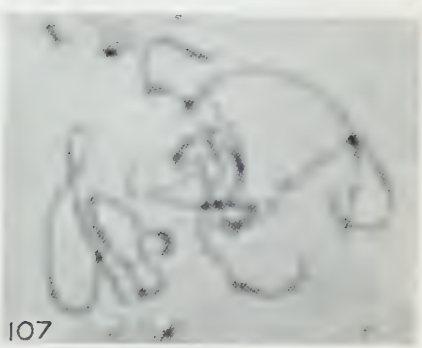


FIGURES 96 to 101. The neural chromosomes of males from: 96, Vittangi; 97, Spindelmuhle 2; 98, Myto 7; 99, Jenikov 1; 100, Padrt; 101, Biely Potok.

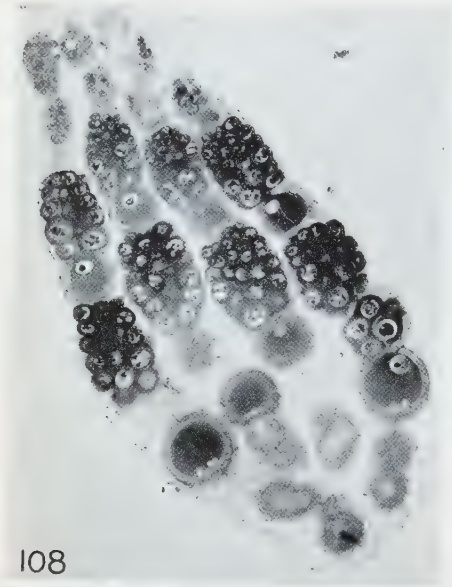


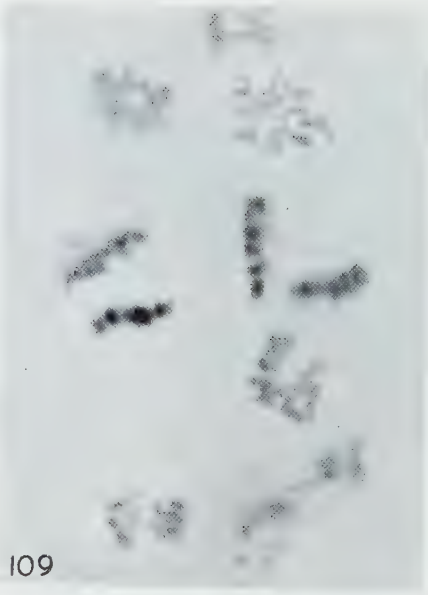


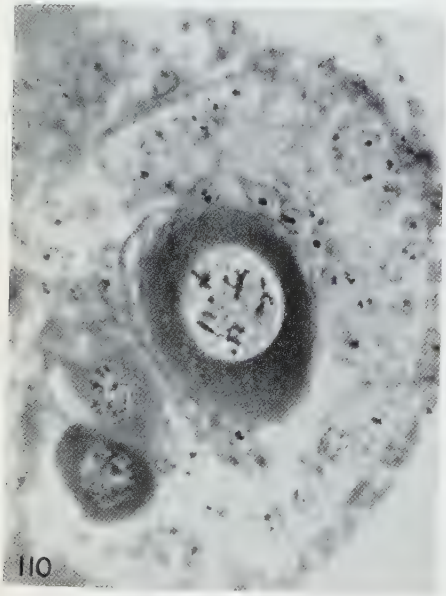


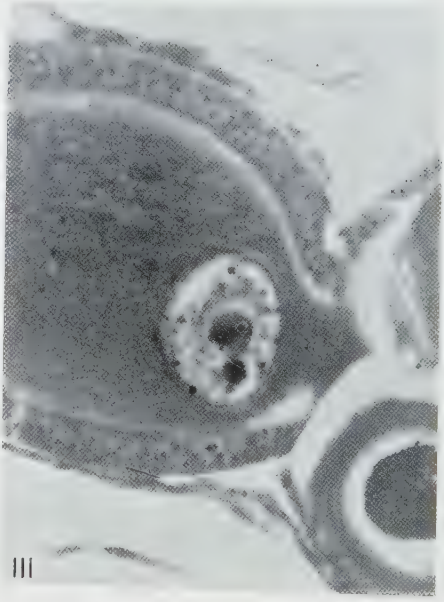


FIGURES 102 to 107. 102, the neural chromosomes of a male from Paltinis. 103 to 106, the somatic chromosomes of females; 103, the obligatory *D. polytomum* from Kout 3; 104, from Klatovy 3; 105, from Canada 2; 106, *D. pallidum*. 107, the pachytene chromosomes of the obligatory *D. polytomum* from Jenikov 5.

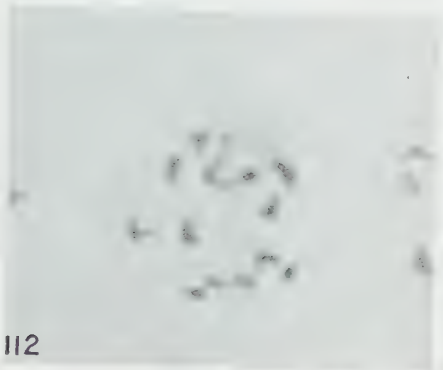


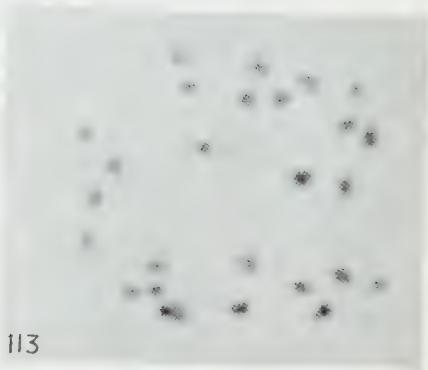


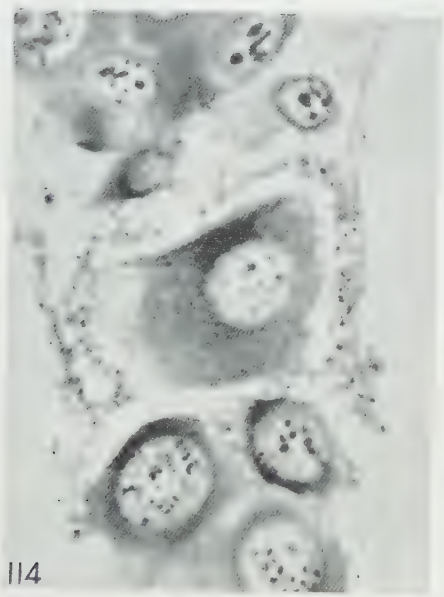




FIGURES 108 to 111. 108, a longitudinal section through the ovaries—Canadian; 109, the 8-cell stage in the oogonial tissue of *D. polytomum* from Myto 10; 110 and 111, the primary oocytes of the Canadian form showing mid diakinesis and the composite body.







FIGURES 112 to 114. 112 and 113, the spermatogonial and oogonial chromosomes of *D. simile*. 114, a longitudinal section through the ovary of *D. simile* showing the diplotene chromosomes in a primary oocyte and the adjacent nurse cells.

FIGURES 115 and 116. 115, the normal bipolar spindles (right) and an abnormal tripolar spindle (left) in secondary spermatocytes of *D. simile*. 116, an anomalous tripolar spindle at the first metaphase in an egg of *D. simile*.

In the early stages, oogenesis corresponds closely with that of other species. Diplotene shows 14 bivalents (Figures 93 and 94) but these have a much higher chiasma frequency than is the case with *D. polytomum*. Despite the numerically tetraploid nature of the oocytes, quadrivalents were never observed. In view of the high chiasma frequency their absence, unlike the failure of bivalent formation in males, may therefore be interpreted as indicating that the species is allotetraploid in constitution.

The maturation divisions of the female have not been studied in detail but at the first anaphase a tripolar spindle was once observed (Figure 116). This is of particular interest inasmuch as 3 examples of tripolar spindles have been seen (Figure 115) among some hundreds of normal second spermatocytes examined, whereas none has been seen in any of the haplo-diploid species. The significance of such tripolar spindles being restricted to the diplo-tetraploid species is not clear at the moment, but it is perhaps in some way related to a possible upset in the timing relationships.

Neodiprion sertifer, Geoffr.

Like the previous 3, this species is a facultative pine sawfly; it occurs freely in the pine forests of Europe. The chromosome numbers for the male and female are seven and 14 respectively (Figure 81) and in both sexes meiosis appears to follow the course normal for facultative Hymenoptera.

Pristiphora erichsoni, Htg.

This species is an obligatory parthenogenetic form feeding on larch. Observations are restricted to the female, though males occur in Canada on rare occasions. The nuclei of the brain show 14 chromosomes (Figure 95) but no satisfactory meiotic figures have been seen.

Comment

The generalization may be made that 7 is the basic number for the genus *Diprion*. There then appears to be no causal relationship between the differences in type of parthenogenesis and chromosome number existing in the 2 forms of *D. polytomum*.

X. DISCUSSION AND CONCLUSIONS

The evidence already presented clearly establishes the fact that in Europe the species *D. polytomum* embraces 2 distinct forms, the one long known and reproducing by facultative parthenogenesis, the other previously unrecognized there and reproducing by obligatory parthenogenesis. Whether the latter which resembles the Canadian form is identical with it is a matter which requires detailed consideration. The difference in the sex of the parthenogenetic progeny is doubtless due to meiosis being normal in the facultative form, as has been shown in analogous cases (Sanderson (38)), while in the obligatory form the first division is similarly reductional but the second compensates for it by the fusion of the telophase nuclei, as has been shown in the Canadian form (Section IV).

From a consideration of the breeding, morphological, and cytological results, it will be seen that the obligatory form has been identified in 18 out of 33 European regions tested but, as the number of individuals

per region examined was frequently small, it is probable that the remaining 15 regions are not all free from this form. Among 14 females tested from Spindelmuhle no obligatory form has been found despite the fact that size selection was practiced on 6 occasions; similarly, none has been identified among the 11 Hradec u Opava females examined cytologically. It is probable therefore that these regions at least are populated only by the facultative form. However, with these exceptions, the obligatory form appears to be distributed freely throughout Bohemia (7 regions) and Slovakia (5 regions) and is present in Moravia and Ruthenia. In addition it has been identified morphologically from Finland and Sweden.

From the limited data available it is naturally difficult to arrive at any conclusion regarding the relative frequencies of the 2 forms in Europe, and particularly so in view of the fact that the majority of the material has been subjected to size selection in order to raise the expected percentage of obligatory females. From the restricted number of random rearings combined with the random cytological examinations there is an indication that the obligatory form might constitute approximately 15% of the collected material, but no estimate can be attempted for Europe as a whole or even for the individual provinces of the Czechoslovakian Republic.

Without exception, the 2 European forms can be further differentiated on the basis of their respective chromosome numbers, the haploid number for the facultative species being 6 and that for the obligatory form 7. The latter is the same as that established for the Canadian form in Section IV. It is to be noted in passing that the occurrence of these numbers (Smith (41)) had not previously been reported for the Hymenoptera, though 7 has since been reported for *Thrinax macula* (Peacock and Sanderson (33)). Based on the disproportionate number of species having a haploid number of 4 or a multiple of 4 (18 out of 22 then examined), Reed (35) was led to suggest ". . . that the series of chromosome numbers in the Hymenoptera may have arisen by polyploidy . . .". Since that time the following numbers have been reported: 1 species with 5 by Gul and Dozorceva (21); 1 with 6 by Smith (41); 7 with 7 by Smith (41); and the present paper and by Peacock and Sanderson (33); 1 with 10 by Dodds (14); 1 with 11 by Speicher (45); and 1 with 14 (Section IX). Thus as opposed to 4 out of 22 we now have 16 out of 34 species which possess a number other than 4 or one of its multiples, and of these 9 are species with a prime basic number. It is clear therefore that there is little evidence for polyploidy in the form of simple multiplication having played a major part in the evolution of the Hymenoptera.

Linear differences are obvious between the somatic chromosomes of various facultative males and between the pachytene chromosomes of various obligatory females. In crosses between certain races and subspecies of *Drosophila pseudo-obscura*, Sturtevant and Dobzhansky (47) have shown a reduction in fertility to be associated with the presence of relative inversions, small deletions and translocations in the chromosomes of the hybrid individuals. Although without recourse to mating it is not possible to determine whether the linear differences between the chromosomes in the 2 forms of *D. polytomum* are due to alterations of this nature, it seems a priori likely that the 2 forms may embrace a number of distinct races. The difference in chromosome number between the 2 parthenogen-

etic forms of *D. polytomum*, together with the obligatory nature of 1 (which constitutes a natural barrier against crossing) are likely to be supplemented by other relative differences in the chromosomes, so that hybrids between the 2 (rare obligatory male with facultative female) even if possible would certainly be more or less infertile. Unfortunately, all crosses attempted have proved unsuccessful. As for the obligatory form itself, in which interbreeding is probably totally inhibited, the conditions are such that mutations must become homozygous, and, recombination being prevented by physiological isolation, the species must sooner or later become split into a number of distinct races.

Morphologically the adults of the 2 European forms have been shown to differ both in the male and the female genitalia. Moreover, it has been conclusively proved that the Canadian form differs from the facultative form in the same way as the European obligatory form does. It is due only to the absence of such taxonomic differences that Races A and B of *D. pseudo-obscura* have not been afforded distinct specific rank. In *D. polytomum* there appear to be adequate grounds for treating the 2 parthenogenetic forms as distinct species.

With regard to other morphological differences, that of gross size promises to be of some importance. Among cocoons selected for large size a total of 44 facultative and 30 obligatory females were identified either cytologically, or by breeding, or by their type of genitalia. On the other hand, random sampling gave 38 facultative and only 4 obligatory females. By means of a fourfold table X^2 is found to have a value of 12.4, which gives a probability of much less than 0.01 that the difference is due to chance. However, no obligatory females were found in the Spindelmuhle and Hradec u Opava collections. If this form is absent from these regions the inclusion of the facultative females will bias the test and they should therefore be excluded. When this is done the remaining figures (41 and 30 vs. 19 and 4) give $X^2 = 4.6$ and P between 0.05 and 0.01, indicating as before that the 2 are distinct as regards size. Since according to this theory selection for small size should increase the incidence of facultative forms, it is almost certain that a comparison of large and small cocoons would supply a greater divergence and consequently a smaller value for P. This was not done, however, as the cocoons of large males and small females frequently overlap in size; on the 3 occasions that female cocoons have been mistaken for male cocoons they have subsequently proved to be facultative individuals.

It has already been shown (Section V) that the mean cocoon weights for the obligatory forms from different regions are statistically different, 2 or possibly 3 being heavier, and 2 the same as the Canadian form. Since these were reared under identical conditions the differences may indicate the existence in Europe of genetically distinct lines. Further, it was stated there that due to the crudeness of the technique employed no significant linear differences were apparent in fifth instar measurements but from the weight differences it is evident that they may exist.

The close resemblance of the obligatory females and facultative males in larval body pattern, despite the difference in sex, is remarkable, for by the time that maturity is reached, the general differences between the 2

sexes is most pronounced. It would appear, from the point of view of the physiology of gene action, that the threshold for the expression of the genes determining the respective secondary sex characters is, like that for the primary sex characters, reached only at the end of larval development. Then, their reaction with the substrate causes a divergence along different lines of sexual development. In view of this close similarity between genotypically distinct forms the extreme differences shown by the larvae of *D. nemorum* and *D. abieticolor*, which have been shown to possess the same chromosome number as the obligatory form, are very striking, and especially so in the case of the latter species which is also closer to *D. polytomum* in adult morphology. We may therefore conclude that even though the obligatory form may present sufficiently distinct physiological and perhaps morphological adult characters to warrant considering it a distinct species, it has not been isolated long enough for morphological differences to develop which will serve to distinguish it in the larval stages from the facultative form.

The red variety of larva was discovered among collections coming from 10 distinct regions in Europe; 1 in Sweden, 1 in Finland, 3 in Bohemia, 2 in the Riesengebirge Mountains, and 1 in each of Moravia, Ruthenia and Roumania. When examined cytologically the numbers were without exception 6 and 12 for the male and female respectively, and when tested parthenogenetically the females were invariably facultative.

In normal, non-parthenogenetic species of insects, recessive genes frequently exist in the heterozygous state, yet the population appears homogeneous because the frequency with which a particular gene occurs is so low that mating between 2 heterozygous individuals is unlikely to occur (Tschetwerikoff (52) and Timofeeff-Ressovsky, H. and N. W., (49) on *Drosophila melanogaster*). To detect such concealed mutant genes in a wild population it is necessary to study the offspring of females collected after mating. Although sex-linked genes and autosomal dominants (introduced at mating) will appear in the immediate progeny, inbreeding for 1 or 2 generations is necessary to disclose the presence of autosomal recessives.

In facultatively parthenogenetic organisms, however, recessive characters, if controlled by a single gene, will show up immediately in approximately half the male progeny because of the absence of dominant alleles in haploid individuals. Further, the frequency of the recessives in the D_2 from facultative forms increases relative to that in non-parthenogenetic forms because all mutants in facultative forms act exactly as sex-linked factors. Thus assuming that an "autosomal" recessive mutation originated in females of both a facultative and non-parthenogenetic species and that these females are then fertilized by their respective wild type (dominant) males, and further that the F_1 progenies, consisting of equal numbers of males and females, intrabreed: from the former there would result a ratio of 9 dominants : 4 heterozygotes : 3 recessives, while from the latter the ratio would be 9 dominants : 6 heterozygotes : 1 recessive. It is to be noted moreover that while the latter ratio is applicable to both sexes of non-parthenogenetic species, in facultative forms, the proportions vary with the sex due to the haploid constitution of the males. Assorted according to sex the above facultative ratio becomes 3 ♀ + 6 ♂ dominants : 4 ♀ + 0 ♂ heterozygotes : 1 ♀ + 2 ♂ recessives.

That red versus green in *D. polytomum* is controlled by a single pair of alleles is indicated by the segregation shown by the heterozygous green females which produced a total of 108 green to 87 red larvae, for by means of the probable error method it is found that the deviation from a 1 : 1 ratio is not statistically significant. Had 2 or more factors been concerned, their phenotypic expression would have been affected according to the presence or absence of dominance (or epistasis). A more conclusive test of the constitution of the heterozygous females would, of course, have been a relatively simple matter if progeny of reciprocal crosses could have been compared, but unfortunately on the few occasions when the appropriate material was available the attempted matings proved unsuccessful.

The difference in cocoon spinning position shown by the two forms is less sharply defined than are most of the other distinguishing characters. The invariability with which the European obligatory form spins its cocoons deep in the moss does, however, distinguish it from the facultative form where the habit is extremely variable, ranging from 0 to 100% in different families from different regions and from 0 to 60% in different families from the same region. Moreover, even those facultative cocoons which are not spun in the foliage differ from the obligatory cocoons in that they are frequently spun on the moss or only just below the surface, whereas the latter are spun as deeply as possible. The European obligatory form thus resembles the Canadian form and is, in fact, more extreme, since in the laboratory the Canadian form occasionally spins in the foliage. In the field, however, it is very unusual to find Canadian cocoons anywhere but in or, if conditions are unsatisfactory, on the ground.

Though the author has no personal experience of the positions in which cocoons are spun in Czechoslovakia, he is informed that they occur there mainly on the bark of spruce trees, in the foliage and on the surrounding herbaceous growth, with, in the colder regions, a small precentage in the surface covering. Whether it is the facultative larvae alone which spin in the foliage, the obligatory larvae invariably spinning in the litter below the tree, is not known. The conditions observed in other species make this possibility very significant. In the laboratory cultures the male progeny of the facultative species *D. abieticolor* have never been observed to spin in the foliage despite the fact that 89 larvae have been reared. On the other hand, larvae of the introduced facultative species *D. simile* spin in the foliage both in the laboratory and the field, where in Canada the temperature frequently drops below −20° F. Monro (32) has shown that sustained low temperatures are lethal to all hibernating larvae spun above the snow line, and that this species is therefore able to survive in Canada only by virtue of those few cocoons which chance to spin in the lower foliage or which have fallen and spun on the ground.

From the parallel evidence obtained from the laboratory and the field in Canada it is clear that the almost invariable habit of spinning in the surface covering shown by the Canadian form is an advantageous character not acquired directly as a response to the more rigorous Canadian climate. Instead it must be regarded as a naturally occurring habit, fortunate for the species (for −22° F. is lethal to all hibernating *D. polytomum* larvae, Balch (1)), and one perhaps essential for its survival since the time of its first appearance in Canada. The protection from the

extremes of the climate and the partial concealment from predators and cocoon parasites afforded by such a position are almost certainly two of a complex of factors which have served to make this form of *D. polytomum* so successful in its invasion of the Canadian forests.

The percentage of larvae which have continued development when removed from cold storage is consistently 100% for all facultative families except for certain of those from Spindelmuhle in the Riesengebirge Mountains. In these latter families diapause affects green and red larvae approximately to the same extent, just as it did their mothers. It is to be noted that the climatic conditions in the Riesengebirge Mountains probably approach those of the Gaspé Peninsula more closely than do those of the majority of the other regions tested. Unfortunately no obligatory forms have been identified from Spindelmuhle so that no direct comparison between the two regions is possible. On the other hand, the obligatory larvae from all other regions except Klatovy have agreed in showing some diapause, but the tendency varies in expression. These regions are all more southerly and may perhaps be compared with the New Brunswick area in Canada where about 70% of the larvae typically develop, though the percentage varies widely from year to year and place to place. All these regions in Czechoslovakia except Brezno are typical two-generation regions, a fact which probably accounts for the direct development noted in the case of 8 males and 10 females in the laboratory. Balch (1) has presented conclusive evidence that the obligatory form in Canada consists of a number of distinct lines varying with respect to their tendency to diapause; whether

TABLE 15.—SUMMARY OF CONCLUSIONS

Parthenogenesis	European		Canadian
	Facultative	Obligatory	Obligatory
Chromosome number ♂	6	7?	7
♀	12	14	14
Morphology—			
♂ — Aedeagus	Spatulate	Pedate	Pedate
♀ — Valvular processes	Straight	Spiralled	Spiralled
♀ — Saw-bands	10	11 to 12	11 to 12
Cocoon spinning position	44% in foliage	0% in foliage	0% in foliage (in field)
Diapause	Atypical	Typical	Typical
Distribution	Widespread in Europe	Possibly somewhat restricted	Restricted to E. of longi. 81° W.
Frequency	*ca* 85%	*ca* 15%	100%
Hypothetical origin	From 7 chromosome prototype by complex chromosomal rearrangement	From 7 chromosome prototype by simple mutation	From Europe by introduction

the same interpretation may be placed on the present results is not clear from the limited amount of data available, but it is certain that diapause is the rule for obligatory forms and the exception for facultative forms in Europe.

The cytological and morphological criteria clearly corroborate the conclusion, arrived at on other grounds, that we are here dealing with two distinct forms, the one facultative and the other obligatory. The virtual absence of males from Canada and their presence in Europe demonstrated this difference without the aid of breeding tests, but because the two parthenogenetic forms overlap in distribution in Europe, at least one of these three criteria is necessary for their identification. The difference in parthenogenesis is in no way a unique condition in the Hymenoptera for of the 2 types 1 is by no means restricted to certain families or even to certain genera. On the contrary, it frequently happens that of 2 related species 1 shows facultative parthenogenesis, the other the obligatory type. This is so with such members of the Tenthredinidae as *Cimbex lutea* (F) and *C. connata* (O); *Eriocampa umbratica* (F) and *E. ovata* (O); and *Pristiphora conjugata* (F) and *P. pallipes* (O). In the ichneumonid, *Hemiteles*, the European species, *H. aerator*, is facultative while the American species, *H. tenellus*, is obligatory. However, no morphological characters have yet been discovered to distinguish between them. The converse is to be seen in the chalcid, *Trichogramma pretiosa*, where the American form is facultative and the European form is obligatory; but in this case the 2 forms have not been elevated to the rank of distinct species. Finally, it may be mentioned that although *T. evanescens* and *T. cecaeciae* differ only in colour, the former is facultative and the latter obligatory.

XI. THEORETICAL CONSIDERATIONS

Vandel (53) discusses in detail the question of the evolution of these two types of reproduction, but this need hardly be dealt with here, except to recognize that the close taxonomic position accorded the above-mentioned Hymenoptera supports the view that the one type is derived from the other. The rare occurrence of males in many obligatory species shows that such species are derived from bisexual species, a conclusion substantiated by the presence of a spermatheca in the female reproductive system.

Although the production of males by obligatory species is a relatively common phenomenon, reports of the production of females by virgin facultative females are extremely rare. Speicher (44) has occasionally induced obligatory parthenogenesis in the facultative parasitic wasp, *Habrobracon juglandis*, by crossing virgin females with unrelated males from distinct stocks. Seven F_1 females from 1 cross were isolated prior to their eclosion; they produced 12 females and 307 males. Further such impaternate females occurred in the F_2 generation from all subsequent crosses between unrelated stocks. That this case is not to be interpreted as a possible way in which the obligatory mode of parthenogenesis can originate or as a demonstration of the close phylogenetic relationship existing between the 2 types of parthenogenesis has recently been shown by K. G. and B. R. Speicher (46). It was considered by Darlington (12, p. 379) as evidence that the second division is regularly reductional for

the X and Y chromosomes, for he assumes quite logically that these diploid females arise by the fusion of the second division products. But it is now found that the impaternate females result from reduction in oocytes which, being derived from tetraploid patches of ovarian tissue, carry the diploid number of bivalents. They thus come to contain the diploid number of chromosomes; it is clear therefore that such induced parthenogenesis cannot be permanent.

Other cases of newly arisen obligatory parthenogenesis, however, seem to be more or less permanent. For example, Paula Hertwig (22) found in a sexual strain of the echinoderm *Rhabditis pellio* a purely parthenogenetic strain which bred true to its newly acquired character. In it there was no change in chromosome number, and at meiosis the oocytes underwent only a single division thereby giving rise to diploid eggs, but the entrance of the sperm was necessary to stimulate development, though fertilization did not take place as in related facultative species. Boveri (6) has shown that in *Rhabditis* the sperm introduces its centrosome into the egg, though no true fertilization occurs, and that following polyspermy the centrosomes of the several sperm develop multipolar spindles. If the conclusion usually drawn from these studies is correct, that the centrosome is transmitted solely by the sperm, it follows that in both facultative and obligatory Hymenoptera, the organizing ability of the egg centrosome must be retained, for reproduction is possible without the intervention of the male. It seems evident that the problem is not as simple as envisaged by early workers. In *Rhabditis* although parthenogenesis must be regarded as a genetic character arisen by mutation, it is, through the necessity for the entrance of the sperm, clearly not self-perpetuating.

A further case of the origin of obligatory parthenogenesis by mutation apparently exists in the wheat stem sawfly, *Cephus cinctus* (Smith (42)). In this species, known for many years as a facultative form presumably introduced into Canada, Farstad (17) has recently discovered a form which reproduces regularly without copulation. Since no obligatory form is known elsewhere and since stimulation by the sperm is unnecessary, this may be considered (*contra Rhabditis*) as a self-perpetuating mutation. If, as is likely, the obligatory form has originated only recently, it is probable that the mating instinct has not yet been lost, so that if males occur it would be possible to investigate the effect of crossing the obligatory and facultative forms to determine whether or not the alternative types of parthenogenesis are inherited as a simple mendelian factor. To the best of the author's knowledge no such information is as yet available.

It would be of interest to know whether chromosome differences exist between the morphologically dissimilar and the morphologically indistinguishable pairs of Hymenoptera mentioned earlier, for that the facultative and obligatory forms of *D. polytomum* can be separated in such a manner has been fully proved in Section VI. Substantiated in some instances by counts obtained from somatic cells, the germinal line nuclei, which are alone concerned with the handing down of the chromosomes from generation to generation, establish the occurrence of a haplo-diploid type of sex determination in both forms.

It will be recalled, however, that in 2 males the spermatocytes were found to be diploid in 1 or a few loculi of the testes, yet no pairing of the

chromosomes occurred. This was interpreted as evidence that the unipolar type of meiosis found in hymenopteran males is genetically determined. In the male desert locust, *Schistocerca gregaria*, White (54) found the normal complement of chromosomes to consist of 4 large, 4 medium and 3 short bivalents, and 1 X chromosome. In 2 nuclei tetraploidy was induced by means of temperature treatment and in the 1 suitable for analysis the chromosomes were present in the form of 4 quadrivalents of large chromosomes, 1 of medium chromosomes, 6 medium bivalents, 6 short bivalents, and 2 unpaired X chromosomes. Because of the absence of trivalents and the restriction of quadrivalent formation mainly to the larger members of the complement, White interprets his results as evidence against Darlington's theory (9) that the formation of bivalents by a tetraploid is the result of the failure of chiasmata to be formed between the 2 bivalents after complete zygotene pairing. Instead he considers that associations of 4 are restricted to the large chromosomes because pairing, which is initially in twos and proceeds along them, is, in smaller pairs, completed before more than 2 chromosomes can come together. However, Moffett (31), working on *Culex pipiens*, found univalents and trivalents in addition to quadrivalents, and pointed out that White's statement is open to criticism; only 1 tetraploid nucleus in *Schistocerca* has been analysed and in other organisms where a number of cells has been examined univalents and trivalents have usually been found at meiosis. More recently, Makino (28) has induced tetraploidy by means of irradiation in *Podisma mikado* (Acrididae) and the normal male complement of 10 bivalents and 1 X chromosome (all with terminal centromeres) was replaced in the large number of nuclei examined by 20 bivalents and 2 unpaired X chromosomes. Trivalents and quadrivalents were never observed. Since the chromosomes in Podisma appear smaller and more uniform, Makino's results seem to support White's conclusion. In both, the failure of the X chromosomes to pair in the tetraploid nucleus is obviously due to precocious condensation (heteropycnosis), a condition not occurring in females. It is clear therefore that, as with the chromosomes in the diploid hymenopteran spermatocytes, the failure of the X chromosomes to form bivalents is genetically controlled and is not directly determined by their normally monosomic constitution.

With the discovery of the X-O type of sex determination it was early concluded that the hymenopteran type of sex determination merely constituted a modification of this. Thus, the male sex in addition to lacking one X chromosome lacks also the remainder of one haploid set, while the female retains the 2 complete haploid complements. With the establishment of the theory of genic balance, however, some doubt was cast on the justifiability of this conclusion, for it was shown that sex is dependent also on the number of autosomes present. More recently the work of P. W. Whiting (58, 59, 60, 61) has shown that this doubt was warranted. He found in the facultative *Habrobracon juglandis* that certain so-called biparental males arise from fertilized eggs, and Torvik-Greb (51) has shown that they contain the diploid number of chromosomes. In endeavouring to reconcile these aberrant males with the conditions usually obtaining in the Hymenoptera, Whiting has been forced to invoke the presence of sex chromosomes in this wasp. By genetic analysis he has produced conclusive evidence for his theory, but it yet remains to prove the existence of

sex chromosomes cytologically. Dozorceva (15) investigating the ichneumon, *Pteromalus puparum*, claims to have identified a pair of heteromorphic sex chromosomes in the female and to have recovered the 2 expected types of males; the one having the larger member of the pair, the other the smaller. But, as Bauer (2) points out, since the males are phenotypically indistinguishable and, we might add, until physiological differences have been established, the size difference may be due simply to one possessing inert chromatin. Their interpretation as sex chromosomes is therefore not necessarily valid.

As some of the chromosomes are sufficiently alike to render their identification uncertain, a close examination of the somatic complements of both forms of *D. polytomum* has failed to yield satisfactory evidence as to whether a heteromorphic pair is present. But at pachytene of meiosis, where the paired condition identifies the homologues with certainty, it is seen directly that no linear differences exist between the synapsed chromosomes. Further, there is no indication of heteropynosis at any stage. It is therefore concluded that in these forms there is no cytological evidence for the existence of sex chromosomes.

In both forms some of the chromosomes of the male and female can be homologized; it now remains to compare the two forms closely to determine whether the extra chromosome of the obligatory form might have arisen in one step by some such method as simple fragmentation, or conversely, whether one of the chromosomes of the facultative form has lost its individuality by fusion. In either case there should be two chromosomes in the obligatory form which together are the equivalent of one large chromosome in the facultative form which is absent from the former. This would perhaps be the simplest explanation for the observed difference, necessitating as it does only the origin or loss of a centromere. That a centromere may be lost is easily imaginable and that a new one can originate by the fragmentation of an old one has been shown by McClintock (29). Recently, Darlington (13)has illustrated the mechanics of the misdivision of the centromere in *Fritillaria*. The morphological evidence, however, clearly indicates a more complex method of origin.

The cytological survey of related species (Section IX) leaves no doubt that the basic chromosome number for the genus is 7, that is, the number possessed by the parthenogenetically atypical form of *D. polytomum*. In view of their close resemblance both in the larval and adult stages, the 2 forms must have been derived from the same prototype and, therefore, 7 must previously have been the haploid number of both. It is possible that the species dichotomy was caused by a unit change initiating the obligatory mode of parthenogenesis for this on its own would prevent crossing with the facultative prototype. Apparently since then, mutation has played only a small part in species differentiation, for the morphological differences between the two forms are very few. The difference in chromosome number is probably the result of a series of complicated alterations which has led to the incorporation of 1 of the 7 chromosomes into the bodies of more than one of the remaining 6. Such alterations are known in Drosophila species. It is surprising only that the change has occurred in the bisexual form, where conditions for its survival are less favourable than in the obligatory form.

The prototype then was probably a facultative form with a basic chromosome number of 7. Presumably it also had genitalia more like Type B than Type A, for Reeks finds that the related species *D. abieticolor*, when classified solely by its genitalia, is easily confused in both sexes with the obligatory form of *D. polytomum*. This opens the very pertinent question as to whether the Ruzomberok family, which morphologically is Type B yet parthenogenetically may be facultative, might not be a representative of the prototype, which therefore would still be extant.

In Section II the various regulatory mechanisms established for obligatory forms were summarized. They will now be considered in respect to their effect on the heterozygosity of the progeny. Since Whiting's theory of sex determination in the Hymenoptera demands that the female sex be heterozygous for the sex chromosomes, it is clear that the theory can be applied to those obligatory forms only in which the regulatory mechanism ensures continued heterozygosity. Where metaphase pairing is absent (Table 16, Types I and II) and meiosis is consequently mitotic in nature, sex-chromosome heterozygosity is necessarily unaltered from one generation to the next; these types therefore conform to the theory. In Types IV, V and VI, however, heterozygosity is maintained only if a chiasma is formed proximal to the X-Y locus; if no proximal chiasma is formed, or more than one are formed in such a way that the alleles separate at the first division, the resulting individual will be homozygous. Using Darlington's (8 and 10) terminology we may say that the pairing segment must be proximal to the differential segment, and, further, that chiasma formation must be such as to ensure post-reduction. In Type III, where the chromatids dissociate at the telophase of the first division and both derivatives undergo segmentation, a proximal pairing segment is again required for the maintenance of heterozygosity. If, however, no proximal chiasma is formed and the alleles separate at the anaphase, mosaics and not pure homozygotes will result. From these considerations it is obvious that Whiting's theory

TABLE 16.—THE EFFECT OF VARIOUS MODIFICATIONS OF MEIOSIS ON HETEROZYGOSITY*

Type	Pachytene pairing	Metaphase pairing	Result	Subsequent division	Genetic constitution of progeny
I	None	None	Equational I	Cleavage	Heterozygosity unaltered
II	None	None	Equational I	Equational II	Heterozygosity unaltered
III	Complete	Complete	Chromatids of Ana I dyads dissociate by telophase I	Cleavage by both derivatives	Heterozygous if alleles fail to separate at anaphase; mosaic if they separate
IV	Complete	Complete	Failure of Ana I	Separation of dyads whose chromatids dissociate at Telo?	Homozygous if alleles separate at first division; heterozygous if at second
V	Complete	Complete	Normal metaphase I	Failure of Ana II	Homozygous if alleles separate at first division; heterozygous if at second
VI	Complete	Complete	Normal metaphase I	Failure of Telo II	Homozygous if alleles separate at first division; heterozygous if at second
VII	Complete	Complete	Normal metaphase I	Normal; cleavage fails	Complete homozygosity

* See pp. 249 and above for details.

is not tenable for all obligatory forms having pachytene and metaphase pairing. Type VII completely invalidates its general extension and the majority demand the existence of differential and pairing segments for which no evidence has so far been presented.

Further evidence that sex chromosomes are lacking from *D. polytomum* is afforded by the occurrence of gynandromorphs in the progeny of Canadian females. As a rule they are antero-posterior, though one was discovered in which only the secondary sexual appendages of the abdomen differed, other appendages and the genital organs being male. Whiting (57) explains the gynandromorphs in Habrobracon as resulting from the fertilization of either the female pronucleus or the second polar body after the accidental retention of the latter. This explanation is not tenable for the obligatory form of *D. polytomum*, as in this species reproduction is effected without recourse to mating. If, instead, the feminized regions are considered to result from a simple doubling of the chromosomes, we again come into conflict with Whiting's theory for, since they must then be homozygous for the sex chromosomes, the diploid regions should be male in character and not female.

From the foregoing considerations it is concluded that Whiting's scheme is specific for Habrobracon and is not to be extended without further evidence to the Hymenoptera in general. Even in Habrobracon the mechanism involving heterogamy co-exists with the haplo-diploid scheme of sex determination, and that their co-ordination is incompletely established is shown by the occasional production of diploid males in inbred stocks. Their occurrence suggests that continual inbreeding, through crossing-over, lessens the difference between the X and Y chromosomes and thus allows similar types of gametes to fuse at fertilization. In other words differential and pairing segments are not developed to the extreme condition.

Balch (1) and others (Prebble, unpublished) have established the fact that under the climatic conditions obtaining in the Gaspé Peninsula the sex ratio is approximately 0.095% males. The emergence records from cocoons collected around Fredericton, N.B., indicate that males may occur more frequently in some of the more southerly regions but the data are variable and rarely conclusive. Thus, although from Douglas Harbour the percentage was 0.376 males among 7,452 individuals recorded, that for English Settlement was only 0.089 among 17,898.

In the laboratory at Fredericton the ratio is considerably higher; based on a total of 4,528 individuals the males constituted about 2.3% of the progeny. At the Farnham House Laboratory the author obtained 3 males among 145 offspring of the European obligatory form, a ratio agreeing closely with that for the Fredericton laboratory. Of 8 Gaspé lines reared at Fredericton and producing 350 offspring only 1 gave a male. The New Brunswick material reared at Fredericton was extremely variable. One line gave 23 ♀♀ : 0 ♂♂; a second 146 ♀♀ : 0 ♂♂; a third 150 ♀♀ : 10 ♂♂; a fourth 3361 ♀♀ : 92 ♂♂; and a fifth 396 ♀♀ : 0 ♂♂. The fourth line was reared continuously for 21 generations during which there was no significant increase in the proportion of males from generation to

generation. However, it is possible that the tendency to produce males is inherited, for selection was not practiced and no increase is therefore expected.

It is to be noted that the frequency with which males are produced is not necessarily a direct index of the frequency with which the regulatory mechanism fails, for should the parent female by heterozygous for a single recessive lethal gene, one-half of her male progeny, being haploid, will be directly eliminated; similarly, one-quarter of her female progeny will be eliminated due to the segregation of recessives. It is possible that the segregation of such lethal genes may be responsible for the difference in fecundity noted between the European obligatory and facultative families reared. The importance of this possibility as a factor in controlling the spruce sawfly in Canada is self-evident, for with three lethals each on a different chromosome the number of offspring would be more than halved.

That a number of distinct lines with varying tendencies to produce males should exist in Canada is not surprising in view of the nature of the regulatory mechanism found in this form. Should the tendency, like that for growth, be controlled by a number of multiple factors, and should the stock originally introduced into Canada have been heterozygous for many of these factors, it is probable that the stock would subsequently become broken up by limited segregation into a number of more or less distinct lines. The process would be a gradual one, each generation in turn contributing something to the building up of complete homozygosity.

Since a high proportion of males has occurred only in laboratory rearings it is possible that the change in sex ratio is correlated with certain changes in environmental factors such as temperature and humidity. The temperature in the laboratory rearings was kept constant at about 72° F., and the relative humidity between 80 and 90%. In all Hymenoptera studied the female pronucleus, as soon as it is formed, has been found to sink into the yolk before undergoing the initial divisions of mitosis. With the type of auto-fertilization found in the obligatory form it seems quite logical to expect that the speed of insinking of the female pronucleus might show a profound difference with variation in temperature. If by raising the temperature, the speed of insinking were accelerated to such a degree that the second polar body were left behind near the periphery of the egg, there would result, not a diploid egg, but a haploid one. In this manner the regulatory process normally incorporated to compensate for the absence of fertilization by the male would not be effective and haploid males would result.

The hypothesis is therefore tentatively suggested that in Canada *D. polytomum* consists of a number of distinct lines as a result of limited segregation in a stock more or less heterozygous for a series of multiple factors each having a tendency to accelerate or decelerate the speed of insinking of the female pronucleus through its influence on the rate of general development. Because of the low temperatures prevailing in the field, the threshold is difficult to exceed, but when the most favourable conditions are provided for an individual with the necessary genetic constitution, the threshold is exceeded and a male results. In the laboratory the constantly higher temperature would lower the genetic threshold to

such an extent that lines which never produce males in the field do so on rare occasions, while those which normally produce a few males now produce them with a higher frequency. Similar multiple factors affecting the rate of development might also explain the existence of diapause lines in Canada.

Some support for this hypothesis is to be found in the general speeding up of larval development by subjecting the larvae to higher temperatures. In the Gaspé (1500 ft. and *ca* 55° F.) 47 days are necessary for development to proceed from hatching to the spinning of the cocoon; at Fredericton (100 ft. and *ca* 67° F.) about 30 days, and in the Farnham House Laboratory (75 to 78° F.) approximately 23 days. Similarly the incubation period varies in length. In the Gaspé at temperatures of 51 to 62° F., the incubation of the egg takes from 24 to 10 days, at Fredericton about 7 days, while in the laboratory only about 5 days are required. Further, in the facultative form in which development may be regarded as being sufficiently rapid to ensure inevitable budding-off, larval development takes only about 15 to 16 days and incubation between 4 and 5 days. Besides being of considerable scientific interest, the production of males is, through its effect on the reproductive rate, an important economic consideration. It is hoped that an opportunity of testing this hypothesis will present itself in the near future.

The production of males in itself lowers the reproductive rate by lowering the frequency with which females are produced, but it is likely to have a secondary influence also. Thus if copulation were common (it has been observed on four occasions in Canada, once in the field and three times in the laboratory) and if fertilization were successful, the resulting eggs should develop into triploid and thus more or less infertile individuals. On 2 occasions progeny have been reared from females observed *in copula* and the sex of the offspring noted. The females in these instances were from the Fredericton high male-producing lines, and the sex ratio ("all to 0 and 11 to 1") deviated in no significant manner from that established for the line as a whole. Moreover, the females appeared indistinguishable from those arising from known virgin females. Unfortunately they were not carried on for a further generation so that it is not known whether they were fertile (diploids) or infertile (triploids), or in other words whether fertilization had occurred or not. That fecundity is lowered in triploid Habrobracon females originating as the progeny of normal females mated with diploid males has been proved by Bostian (4). Egg production was found to be about one-fourth that of normal diploid females, while ". . . . . hatchability of eggs and production of adult offspring were, respectively, 1/50 and 1/800 that of the diploid females." Since the triploid females have been shown (Whiting, A. R. (55 and 56) and Torvik (50)) frequently to be morphologically abnormal, it appears unlikely that fertilization occurred in *D. polytomum*.

Since the obligatory form most likely arose from a bisexual form it is feasible to assume that it was more or less heterozygous when it originated. Physiological evidence indicates that the Canadian form consists of a number of distinct lines. This suggests that the stock was heterozygous when first introduced into Canada, for considering the effect of diapause

in lowering the reproductive rate it has probably been here too few generations for new mutations to be responsible for the observed diversity. If this is so, there were still heterozygous individuals in Europe at the time it was introduced into Canada. Since the regulatory mechanism inevitably increases homozygosity, such recent heterozygosity in Europe, together with its possibly restricted distribution there, suggests that the obligatory form is of recent origin. Some supporting evidence is possibly provided by the Ruzomberok family and by the occurrence of a 7-chromosome male in a collection of *D. polytomum* from Rukatunturi, Finland, for these may be representatives of the hypothetical prototype.

Regardless of when and by what means the obligatory form arose it is clear from the accumulated evidence (see Table 15) that the obligatory form differs sufficiently from the facultative form to merit raising it to distinct specific rank. A description of the morphological differences will be published shortly by W. A. Reeks who will propose a name for the obligatory form.

XII. SUMMARY

The bionomics of the spruce sawfly, *Diprion polytomum*, first discovered in the Gaspé Peninsula in 1930, is outlined in Section I and compared with that of the form originally recognised in Europe. The two appeared morphologically similar but differed as follows: (1) in Canada parthenogenesis is obligatory; in Europe it is facultative; (2) in Canada the cocoons are almost invariably spun in the surface covering; in Europe the lower foliage and the surrounding herbaceous growth are usually preferred; (3) a varying percentage of the Canadian cocoons remain dormant (diapause) for one or several years; in Europe two generations per year are usually produced. These differences suggested a comparative cytological examination to discover an explanation for the difference in parthenogenesis and to establish the identity of the Canadian form.

In Section II the cytology of parthenogenesis is briefly reviewed and in Section III the cytological technique employed is outlined.

The results of the preliminary cytological study are given in Section IV. Canadian females and males (rare) possess 14 and 7 chromosomes respectively, while a collection (presumably facultative) from Hradec u Opava, C.S.R., consisted of females and males with 12 and 6 chromosomes each. Sex determination in both is thus of the haplo-diploid type typical of the Hymenoptera. In males from both countries the first meiotic anaphase is abortive, and spermatogenesis involves no reduction in chromosome number. In females all stages of meiosis examined were normal. Chromosome reduction in the Canadian eggs is compensated for by the re-fusion of the second polar body with the female pronucleus; the eggs thus retain the diploid number of chromosomes and give rise to females. That the regulatory mechanism may fail is shown by the rare occurrence of males (which have the haploid number of chromosomes); these constitute about 0.09 to 0.38% of the field population. A single female from Albrechtice, Silesia, was found with 14 chromosomes, which indicated the existence there of a form hitherto unsuspected.

To test this possibility 15 collections were obtained from different regions in Czechoslovakia and progeny were raised from virgin females (Section V). The females were found to be of two parthenogenetic types, the one facultative and the other obligatory, but the status of one female from Ruzomberok was questionable. The former was identified from all remaining regions; the latter from only 6. The percentage of cocoons spun in the moss differed for the various facultative families, but no obligatory larva was found elsewhere. Diapause was typical for the obligatory form but occurred in facultative families from one region only. Comparative rearings were made from Canadian females and those of two other species.

A correlation of parthenogenesis, chromosome number, and adult morphology was undertaken independently by the author and W. A. Reeks. All facultative males possess 6 chromosomes and all obligatory females have 14 (Section VI). Rare diploid spermatocytes retain the unipolar type of spermatogenesis. Measurements at pachytene in the oocytes indicate that there may be real differences between the chromosomes of females from different localities.

Morphologically the facultative females differ from the obligatory females in having 10 instead of 11 or 12 saw-bands on the first pair of valvulae and straight instead of spiralled processes on the second pair (Section VII). The facultative male progeny differ from the only available obligatory male in the shape of the intromittent organ. In contrast obligatory females and males from Canada are identical with the European obligatory form. An aberrant Ruzomberok female with obligatory type genitalia produced a single male offspring also with obligatory type genitalia; her status is therefore uncertain, and the possibility is considered that she may belong to the putative prototype.

Using chromosome number and adult morphology as criteria, 19 miscellaneous collections were examined from Sweden, Finland, Roumania and the 4 Provinces of Czechoslovakia to determine the distribution of the obligatory form in Europe. (Section VIII). It is concluded that the obligatory form is less widely distributed in Europe, possibly being absent from some regions in northern Bohemia and Moravia.

To determine whether 6 or 7 is the basic number for the genus, the chromosomes of 6 related species were examined (Section IX); 5 had 7/14 and 1 had 14/28 chromosomes. It is concluded that the obligatory habit arose independently of numerical change.

From the evidence presented it is concluded that the European obligatory form and the Canadian form are identical; the latter originated in Canada by introduction.

The occurrence of a mutant larval type confined to the facultative form is recorded, and its relatively high frequency is discussed in relation to the fact that all mutations in facultative forms act precisely as sex-linked genes.

The bearing that these results have on the general applicability of Whiting's scheme of sex determination in Habrobracon is discussed. It is concluded from a consideration of the various regulatory mechanisms

established for obligatory forms that in Habrobracon sex chromosomes are newly evolved and incompletely co-ordinated with the haplo-diploid type of sex determination characteristic of the Hymenoptera.

A hypothesis based on temperature effect and rate of development determined by multiple factors is tentatively presented to explain the natural occurrence of males in the progeny of the obligatory form and their greater frequency in certain laboratory rearings.

That the difference in fecundity of European obligatory and facultative forms could be due to the presence of recessive lethal genes in the former is shown and the effect their presence would have in controlling the outbreak in Canada is briefly discussed.

XIII. ACKNOWLEDGMENTS

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BOOK REVIEW

"The Scientific Principles of Plant Protection with Special Reference to Chemical Control" by Hubert Martin, D.Sc., etc.; 3rd edition 1940; page 1–VII, 1–385 with author index and subject index. Edward Arnold and Company, London, (Eng.). Price 22/6 ($3.75).

The first edition of this book appeared in 1928, this was followed by a second in 1936 and the present edition has been necessary to include the many developments which have taken place in plant protection during the past four years. In order to include this mass of material the book has been reset and the text drastically pruned. As is indicated by the title special emphasis is laid on chemical protection and five of the sixteen chapters are devoted to the history and development of insecticides and fungicides. Besides an introduction the other chapters cover such subjects as plant resistance, the influence of external factors on the susceptibility and liability of the plant to attack, biological control, weed killers, fumigants, seed treatment, soil treatment, toxic action and chemical constitution, traps, and the treatment of the centres and vectors of infection.

The author points out that plant protection involves not only biological but equally important chemical and physical studies and emphasizes the necessity of the closest co-operation between the entomologist, mycologist, chemist, physicist and plant physiologist. He refers to the fact that "as successful control is based only upon accurate knowledge of the life history and habits of the organism responsible, the advisory officer is rightly recruited from the biologists. He is, therefore, better acquainted with the biological than with the physico-chemical aspects of Plant Protection." For this reason, greater emphasis is laid on the latter aspects in this volume but this is not meant to indicate that they are of more importance than biological or cultural methods of control. This book is also intended to provide a reference on insecticides and fungicides, "the chemistry of these materials is a young science".

In the preparation of this volume the author has shown painstaking effort. In accumulating the vast number of references acknowledgment is made of "the value of the abstracts now provided by the Imperial Bureaux of Entomology, of Mycology, and of Horticulture and Plantation Crops". He is to be congratulated on his clarity and conciseness as well as for the general arrangement of the text. The references at the end of each subheading in the various chapters, the full subject index covering thirty pages and the author index of twelve pages are very helpful and useful to the reader.

Specialists in various subjects may find omissions, these are bound to occur in a work such as this. For instance it is to be regretted that under the chapter fumigants no reference is made to some of the newer chemicals, particularly methyl bromide, and the same also applies to soil treatments. However, this volume should commend itself to all those interested in plant protection, irrespective of the special field in which they may be engaged as it is a comprehensive work and makes interesting reading.

—Leonard S. McLaine.